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CONTENTS

BOSCHMA, H. *On the Feeding Reactions and Digestion in the Coral Polyp Astrangia danæ, with Notes on its Symbiosis with Zooxanthellæ* 407

CLARE, M. R. *A Study of Oxygen Metabolism in Drosophila melanogaster* 440

HEILBRUNN, L. V. *The Action of Ether on Protoplasm* 461

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BIOLOGICAL BULLETIN

ON THE FEEDING REACTIONS AND DIGESTION IN THE CORAL POLYP *ASTRANGIA DANÆ*, WITH NOTES ON ITS SYMBIOSIS WITH ZOÖXANTHELLÆ.

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In comparison with the extensive literature on the feeding reactions and the digestion in actinians, these phenomena in madreporarian polyps have been studied by a few authors only. Our knowledge of these phenomena is chiefly due to Carlgren (1905, *Caryophyllia*), Duerden (1906, *Fungia* and *Favia*), Carpenter (1910, *Isophyllia*), and Vaughan (1912, various West Indian reef-corals). In the following pages the results are given of an investigation on the feeding reactions and the digestion in the coral-polyp *Astrangia danæ* Ag.

The work on which this paper is based was made possible by a subvention of the Netherland-America Foundation, which enabled me to visit the United States for some months. It was carried on in the Marine Biological Laboratory at Woods Hole (Mass.) in August and September, 1924. I want to thank here the Director of this Laboratory, Dr. F. R. Lillie, for the opportunity I obtained to work some time at this institution.

The study of the digestion in *Astrangia* is meant as a base for the further study on the digestion in the polyps of reef-corals. As is well known, the polyps of reef-corals contain a large number of unicellular algæ, the zoöxanthellæ, which are located in the entoderm-cells. Although this association of coral-polyps with algæ is generally considered as a kind of symbiosis, there are very few statements on the advantages which both organisms derive from it. One of the ways to investigate this problem is the comparison of the physiology of a form with symbiotic algæ with

that of a form without these organisms. Now *Astrangia danæ* generally is completely devoid of zoöxanthellæ, but during the time I worked in Woods Hole I obtained some colonies which were strongly infected with these algæ. The fact that my material consisted partly of polyps without zoöxanthellæ and partly of those infected with the symbiotic algæ enabled me to make a comparison of the feeding and digestion in these different kinds of polyps. As I disposed during the first half of my time in the Marine Biological Laboratory only of polyps without zoöxanthellæ, the larger part of my experiments were made with such uninfected polyps. Afterwards, when comparing the phenomena of digestion in polyps without zoöxanthellæ with those found in uninfected polyps, I could at least partly investigate the rôle of the zoöxanthellæ in the feeding of the polyps which are infected by these algæ.

I kept the polyps of *Astrangia* easily alive in glass-vessels with running sea-water, and at the end of a month they were in the same healthy state as at the beginning of the experiments. Some colonies even had enlarged in size by budding. The fact that *Astrangia* may be kept alive for some time in artificial surroundings was already recognized by Agassiz (1850), who kept his specimens alive by changing the sea-water twice a day. This author also gives a description of the structure of the polyps and states that they can be fed with fragments of molluscs. Besides this he gives some notes on the digestion in *Astrangia*. According to Agassiz the food at first remains for some time in the upper part of the gastric cavity before it descends to the lower part of this cavity where it is mixed with water and distributed over the internal organs of the polyp. As may be seen in the following pages these notes on the digestion are not completely in accordance with my observations.

The description of the general form of the polyps by Agassiz is very accurate. He states that there are three cycles of tentacles, those of the first cycle are of a larger size than those of the second, whilst the tentacles of the third cycle are somewhat smaller than the others. At each extremity of the laterally compressed mouth a tentacle of the first cycle is found. Agassiz discerned two varieties in the species: one with white polyps and another in which the polyps were pink or rosy.

An account of the chief peculiarities of *Astrangia danæ*, accompanied by figures, some of which represent the polyps in an expanded form, are found in the publications of Mrs. and Mr. Agassiz (1865) and of Dana (1890). Verrill and Smith (1874) also give a short description of the polyps of this species. They state that the animals are white and that the polyps in expansion rise high above the skeleton. These authors also note that the polyps will feed readily upon fragments of molluscs or crustaceans.

In the literature on the coral *Astrangia* I have not found any remark on its symbiosis with zoöxanthellæ. In the greater part of the colonies of this species found in the Woods Hole region all polyps are completely free of zoöxanthellæ. This was invariably the case in the specimens which I obtained from the piles of the wharf at Woods Hole, in those dredged in the harbour between Woods Hole and Nonamesset Island and in those dredged to the southeast of Nonamesset Island from about 8 fathoms. On the other hand the specimens dredged to the southwest of Falmouth (south of the oyster-pond) were always more or less infected with zoöxanthellæ. Some of the polyps of this locality had a dark brown color owing to the multitude of yellow algæ occurring in their tissues, others had various lighter shades of brown or were almost white, but a microscopical examination of a part of their tentacles or oral disk proved that they invariably contained zoöxanthellæ. It is a strange fact that this symbiosis of the polyp with yellow cells in the Woods Hole region is restricted only to the colonies found in certain smaller localities.

The polyps which are not infected by zoöxanthellæ are quite colorless and these are therefore especially fit for the study of the feeding reactions as foreign bodies can be seen through the transparent tissues of the animals. Usually the skeleton has a greenish or light-red color, which is due to the occurrence of algæ living in the skeleton. Then at first sight the polyps sometimes seem to have a pink or greenish hue by the transparency of their tissues. Probably these red or green-colored algæ are only different stages in the development of one species as their microscopical structure is very much alike. As yet I have no positive evidence that they belong to one of the forms described by Duerden (1905). In some colonies a compact mass of algal matter is obtained after

decalcifying the specimens, in other colonies only sparsely distributed threads are to be found. In an allied species, *Astrangia solitaria*, the skeleton is also penetrated by boring algæ (Duerden, 1902). Besides these boring forms there is another alga which lives in the tissue of the polyps. Mrs. Dr. A. Weber van Bosse, who kindly examined the preserved material of these algæ, found that it represents a new species of the genus *Streblonema*.[1]

For different reasons the polyps of *Astrangia* are especially fit objects for the study of their feeding habits. During the daytime they are usually fairly well expanded.[2] After being disturbed the polyps contract within the skeleton, but usually they will expand again in a very short time after the disturbance. Even when they are transferred from one glass-vessel to another they soon return to their expanded state. One of the further advantages of *Astrangia* in my experiments was that the polyps will ingest almost every particle which is offered to them, as well food as non-nutrient objects.

All the polyps of freshly-collected colonies of *Astrangia danæ* do not react in quite the same way on a certain stimulus, probably on account of the disturbance caused when they are collected. When, however, the colonies have been a few days in the laboratory under constant external influences they constitute a fairly uniform material for feeding experiments. Especially more or

[1] I am indebted to Mrs. Dr. A. Weber van Bosse for the following diagnosis of this interesting form:

"*Streblonema Willyae* n. sp.

Frondibus microscopicis in telum *Astrangiae danae* penetrantibus, compositis e filamentis sterilibus, irregulariter alterne aut secundatis ramosis, 2–5 μ latis, aggregatis, fasciculos prope superficiem hospitis formantibus. Chromatophoris taeniatis aut disciformibus, parietem cellulae non totius tegentibus. Sporangiis ignotis. Gametangiis cylindricis aut fusiformibus, singulis aut ramosis in filamentis plerumque terminalibus, interdum lateralibus, longis 60–120 μ, latis 8–10 μ; loculis uni- et pluriseriatis. Pilis desunt.

This new species of *Streblonema* is so far interesting as it grows in a coral, not in the skeleton but in the soft tissue of the animal. It has this mode of living in an animal in common with the genus *Endodictyon*, but the apical and above all the branched gametangia seem to indicate that its proper place is in the genus *Streblonema*."

[2] It is a well-known fact that the greater part of the madreporarian coral polyps are nearly always found in a contracted state during the day-time. Besides *Astrangia* there are, however, exceptions on this rule, *e.g.*, the polyps of *Fungia*, *Goniopora* and *Euphyllia* from the East Indian coral reefs, which are during daytime in a fully expanded state.

less hungry polyps are very susceptible to a certain stimulus, as is the rule in anthozoans. On the contrary well-fed polyps react very slowly on the same stimulus. Also in this respect *Astrangia* agrees with other anthozoans (cf. Jennings, 1905).

The chief results of my experiments on *Astrangia* are published in a preliminary paper (Boschma, 1925), in which especially the rôle of the zoöxanthellæ in the feeding of the polyps is compared with that in other anthozoans.

Feeding Reactions.

The reactions of *Astrangia* to food and other substances are in many respects quite similar to those found in other representatives of the anthozoans. Before the results of my own investigations on *Astrangia* a short summary of the literature on this subject is given below.

Pollock (1883) was the first to describe that sea-anemones may perceive the presence of food-particles in their neighborhood, as this causes the opening of the mouth and movements of the tentacles of the animals. Loeb and Nagel some years afterwards worked out the reactions to food and other substances in actinians more in detail. According to Nagel (1892, 1894*a*, 1894*b*) the actinians have a sense of taste, which is located only in the tentacles, and the food is brought to the stomodæum by muscle-action. Nagel (1894*b*) already stated that hungry actinians also ingest morsels of paper besides food-particles, whereas well-fed ones will only take food. The results of Loeb (1891, 1895) differed in some respects from those of Nagel. The phenomenon which was called by Nagel the "sense of taste" is according to Loeb a reaction to chemical stimuli. Loeb showed that the tentacles are not the only organs in which the response to chemical stimuli is located, he stated that also ciliary actions plays a part in the feeding of actinians. Moreover by cutting an actinian into two halves Loeb proved that a part of such a polyp devoid of its tentacles even takes food more readily than a normal individual.

Parker (1896) confirmed Loeb's results that ciliary action is one of the chief factors in the feeding of actinians. Parker found that the cilia of the stomodæum generally beat outwards, but when food substances come into contact with these cilia they reverse

their movement. In subsequent papers (Parker, 1905*a*, 1905*b*) the same author has worked out this phenomenon in further details. He found that certain chemical substances can induce the cilia to reverse. A number of statements on the part of the tentacles and of the ciliary action in different actinians during the feeding reactions is described by Carlgren (1905). This author found that in some species of actinians the ingestion of food takes place chiefly by ciliary action (*e.g.* in the Protantheæ); in other forms, however, the tentacles are the chief organs for the capture and transportation of the food to the mouth (*e.g. Tealia*).

The fact that not all the individuals of a certain species of actinian react in the same way on the same stimulus, was demonstrated by Jennings (1905). The state of metabolism of the actinian is one of the most important factors determining the reaction to substances which come into contact with the tentacles or the oral disk. Thus hungry polyps often will ingest inedible matter, while well-fed ones will refuse the same material. Piéron (1906) also states that different individuals of one species behave differently towards the same stimulus.

Different species of actinians also behave in quite a different way. Some species of actinians in confinement would ingest only a very limited variety of food, *e.g. Eloactis*, which refused everything but living *Balanoglossus* and *Hydroides* (Hargitt, 1907). On the other hand many other species ingest besides food also indigestible material. Instances of this are recorded by Torrey (1905) for *Sagartia*, Fleure and Walton (1907) for *Tealia*, Parker (1896) and Allabach (1905) for *Metridium*. In the case of *Metridium*, Parker (1905), however, has expressed the opinion that the foreign matter (filter paper) was touched by the hands of the experimenter and therefore acted as a chemical stimulus. All of the above-named forms, as also *Actinia* (cf. van der Ghinst, 1906) are able to discern food-particles from inedible matter. The latter is usually refused and food particles are ingested.

Summarizing the data available in the literature and those of his own investigations Parker (1917) states that the different factors which are combined to bring about the feeding reactions in actinians are the following: secretion of mucus, ciliary action, the action of the neuromuscular apparatus of the tentacles, of the

œsophageal cilia, and of the transverse mesenteric muscles. Only the muscular reactions are under the control of the animal as a whole.

The feeding reactions of *Alcyonium* are described by Pratt (1906). In this form the tentacles are the chief organs for the capture of the food. The species exhibits a definite choice in selection of the food, as eggs of fish and of *Galathea* are not digested, whilst the polyps readily feed upon planktonic organisms and flesh of different fishes.

There are only a few papers dealing with the feeding reactions in madreporarian polyps. The first species, in which observations on the capture of the prey are recorded, is *Astrangia danæ*. Verrill and Smith (1874) mention that it catches its food with the tentacles, which afterwards transfer the food to the mouth. De Lacaze-Duthiers (1877) made some feeding experiments with *Caryophyllia Smithii* and *Balanophyllia regia*. In the former species the food, a piece of a living mollusk, placed on the oral disk, caused a depression of the disk in this place. By the action of the muscles of the oral disk it was now brought to the mouth. The tentacles remained quite inactive. In *Balanophyllia* on the contrary the tentacles pushed the food towards the mouth. After some time in both species the food was discharged through the mouth covered with mucus. These statements, however, can hardly represent the normal behavior of the polyps. Probably the animals were in an abnormal state, for De Lacaze-Duthiers had kept them during several years in captivity. Moreover these statements are not in accordance with those by Carlgren (1905) on *Caryophyllia*. According to Carlgren the tentacles of this species catch food-particles and deposit them on the central part of the oral disk. By ciliary movement they now are transported over the oral disk to the stomodæum and swallowed.

The polyps of *Siderastrea radians* seize the food, according to Duerden (1904), with the tentacles. According to the same author (Duerden, 1905) mucus plays an important part in the feeding reactions of *Fungia* and *Favia*. On the surface of the polyps a mucous layer is secreted. Small organisms which come into contact with the oral surface of the polyps are imbedded in the mucus, which is afterwards ingested.

Carpenter (1910) described the feeding reactions of *Isophyllia*. The tentacles of this species catch small planktonic organisms. When a certain amount of food is taken by the tentacles the sphincter of the edge-zone of the oral disk contracts and the oral disk then forms a kind of roof over the mouth and the surrounding parts. In the superficial chamber formed in this way the digestion of the food takes place.

Vaughan (1912, 1919) studied the reactions to food in *Mœandra areolata* and many other West Indian reef-corals. The food is ingested through the combined action of ciliary movements, secretion of mucus, and the movements of the tentacles. In some species, *e.g.*, in *Orbicella cavernosa*, the mesenterial filaments are often protruded through the column wall and can catch food and even digest it whilst remaining outside of the gastric cavity.

With my feeding experiments on *Astrangia* I could confirm the statements of former authors that it will readily take food in captivity. When not overfed the polyps even will ingest almost everything which is offered to them. The food-objects which I used in my experiments were the following: crab meat (the muscles of the legs of the spider crab, *Libinia caniculata* Say.), fish meat, the soft parts of mussels, different species of worms (*e.g.*, *Naraganseta coralii* Leidy which lives burrowing in the skeleton of *Astrangia*, *Hydroides* and other polychæt worms), pycnogonids, small amphipods, larvæ of higher crustaceans, copepods, ctenophores, etc. Also juice of crab or mussel meat is readily ingested. Besides these food-particles indigestible objects are ingested by hungry polyps with the same avidity: coarse sand, powdered writing chalk soaked in sea-water, clumps of litmus, carmine and iron carbonate.

When crab meat or fish meat is mixed with some coloring matter (India ink, litmus, ammoniac carmine, neutral red) it is as readily taken as pure meat. This method enabled me to trace the way of the food in the internal organs after it had been ingested, the coloring matter was only used as an indicator.

These experiments with pieces of meat could not give results which represent the normal feeding reactions. In the gastric cavity of freshly collected specimens often remains of small organisms are found, especially appendages of small crustaceans.

To study the normal feeding reactions I therefore fed the polyps with planktonic organisms, chiefly copepods and larvæ of decapod crustaceans. It proved to be an advantage when the food was colored, then it could be seen through the transparent tissues when it was captured and swallowed. For this reason I used the method employed by Fischel (1908) for daphnids and copepods of fresh water. To the sea-water which contained the animals a few drops of a solution of neutral red were added by means of which the water obtained a light yellow color. The copepods and other organisms now absorbed the coloring matter and gradually acquired a bright red hue. Especially the different parts of the intestinal tract absorbed a great quantity of neutral red, but also immediately beneath the skeleton and in the appendages the coloring matter was stored. These colored copepods and larvæ of decapods are as readily taken by the polyps as colorless ones.

When some of these colored planktonic organisms are put into a small glass containing a colony of *Astrangia* with expanded polyps the capture of these animals may be easily observed. Every now and then one of the animals comes into contact with a tentacle of a polyp. Smaller copepods then as a rule are immediately captured, they seem to stick to the tentacle, undoubtedly by their being paralyzed by nematocysts. The tentacle then suddenly contracts more or less and bends over the oral disk in the direction of the mouth. The prey, however, is not brought directly to the mouth. The latter, with the central part of the oral disk, slowly increases in height as a conical protusion and this expanded part gradually moves in the direction of the prey. At last the tentacle with the copepod comes into contact with the mouth, the tentacle releases the prey and bends back to its original place. The captured copepod slowly slides down through the stomodæum into the gastric cavity, undoubtedly by ciliary action, and the mouth returns to the central part of the disk.

Larger copepods and larvæ of decapods, however, are not so easily captured. When they touch a tentacle they often swim away with a sudden jerk, the attack of the nematocysts seeming insufficient to paralyze them. Only when these animals bump heavily against a tentacle they are immediately caught. Their

struggling movements cause a number of neighboring tentacles to move towards them, so that they finally become completely enveloped in a number of tentacles. Then they are slowly transferred to the mouth in the above described way, but they often remain struggling heavily, even when going down the stomodæum. Usually, however, the tentacles keep these larger organisms in the same position for some time, and only after they have ceased to struggle they are transferred to the mouth.

These experiments with copepods and other small crustaceans show that the tentacles instantly react when they are touched by a free swimming animal. The oral disk on the contrary is quite indifferent to the contact of these planktonic organisms. Sometimes a small floating copepod may be seen sinking down and falling on the oral disk of a coral polyp. It can remain there for some time without calling forth any reaction of the polyp. Afterwards it can swim away unharmed unless it happens to touch one of the tentacles, in which case it immediately brings forth the capturing reactions of the tentacle.

In the case of the feeding experiments with crab meat or other non-moving material of food the movements of the tentacles are very slow. In expanded polyps the small morsels of meat strongly adhere to the tentacles to which it is offered, and usually some neighboring tentacles also bend towards the meat, more or less enveloping it. The mouth with the top of the conically expanded central part of the oral disk moves slowly towards the prey and the tentacles push the meat downwards into the stomodæum. Some tentacles often protrude into the stomodæum, pushing against the food-material. The mouth then closes and the food slowly passes down into the gastric cavity.

Generally after the feeding the polyps partly expand by raising their column often considerably above the skeleton. The tentacles also remain in a more or less expanded condition. This expansion of the polyps generally takes place as well after the feeding of meat as after the feeding of free-swimming organisms. In this state the polyps are almost indifferent to mechanical stimuli. Whilst a hungry polyp quickly contracts when gently touched by a forceps the polyps which have just fed do not react on the same stimulus. A further peculiarity is that often some

time after feeding air-bubbles are to be seen in the upper parts of the polyps, probably escaping from the prey which is being dissolved in the gastric cavity.

The reactions of hungry polyps of *Astrangia* to comparatively heavy objects, as diminutive pebbles and clumps of litmus, writing chalk or iron carbonate, are almost the same. These objects, when laid against a tentacle or on the oral disk, bring about the feeding reaction, consisting of the above described movements of the tentacles and the central part of the oral disk. The objects are almost invariably ingested and come closely in contact with the mesenterial filaments. The mouth closes after the ingestion and the polyp remains for some time, in my experiments varying between 20 and 95 minutes (usually lasting about 50 minutes), quite motionless. The first movement made after this time usually is the enlargement of the central part of the oral disk to a conical protusion. Then the mouth opens and the foreign object is seen moving slowly upwards in the gastric cavity. The underside of the object is in touch with the mesenterial filaments of the polyp, which seem to push against it. In the stomodæum in all probability the outward movement of the foreign object is caused by ciliary action, but often some mesenterial filaments are seen protruding in the stomadæum till the object has been removed from the inside of the polyp. In this way the non-nutrient paticles are soon out of the gastric cavity, chiefly by the action of the mesenterial filaments. The object falls from the mouth on the oral disk and slides down on one side. Here the tentacles bend downwards and then the object is completely removed from the polyp.

The foreign objects which have been in the gastric cavity for some time are covered with a thin mucous layer. Undoubtedly the mucus has here a protective function, to avoid that noxious particles come into contact with the tissues of the polyp. This function of the mucus is already described by Gee (1913) in *Cribrina*. In *Astrangia* especially on the clumps of chalk which are removed from the gastric cavity the mucus is clearly visible. Often other particles imbedded in this layer are removed with the non-nutrient object. In this way very small quantities of remains of food may be obtained from the gastric cavity without

causing any injury to the polyp. I used this method to make out whether or not zoöxanthellæ were present in the gastric cavity of polyps the tissues of which contained these unicellular algæ.

Small pieces of filter paper rarely induce the polyps of *Astrangia* to feeding reactions. To avoid the absorption of organic substances (cf. Parker, 1905) the pieces of paper were not touched with the hands, but they were handled by clean instruments and put on the tentacles of a hungry polyp. For some time the small objects remain attached to the tentacles, to which they adhere more or less, but as a rule they soon fall down without causing the feeding reaction. In some cases, however, a few pieces of filter paper are ingested and afterwards, after about 50 minutes, removed from the gastric cavity in the above described manner. The difference in behavior of hungry polyps to comparatively heavy objects as small pebbles and pieces of filter paper in all probability is caused by the difference in weight. The heavy objects give a definite mechanical stimulus which immediately brings forth the feeding reaction, whilst the pieces of filter paper act as indifferent objects.

When carmine-powder soaked in seawater is distributed over expanded hungry polyps large quantities are ingested. There is no evidence of ciliary action on the oral disk, probably cilia are not to be found here. Soon after the carmine has dropped on the oral disk it is imbedded in mucus and large strains of this mixture of mucus and carmine can be seen gliding down the stomodæum through the inward beating of the cilia of the latter. Often a quantity of carmine is ingested by the cells of the mesenterial filaments, but it also occurs, especially when large quantities of carmine are present in the gastric cavity, that clumps of carmine mixed with mucus are removed from this cavity as non-nutrient particles.

The feeding reactions of *Astrangia* in general therefore consist chiefly of four actions of the polyps: muscular action of the tentacles, muscular action of the central part of the oral disk, secretion of mucus by the oral disk, and ciliary action of the stomodæum.

Digestion.

In a great number of anthozoans, chiefly in actinians, the phenomena of digestion are at least partially investigated. The different authors who have worked on this subject do not agree in every detail, though the chief features of the digestion are fairly well known.

The principles of our knowledge of the phenomena of digestion in actinians and other cœlenterates are chiefly due to Metschnikoff and Krukenberg. Metschnikoff (1880, 1882) found that small food-particles are ingested by the entoderm cells of the mesenterial filaments in an amœboid way. They are imbedded in these cells and digested here (intracellular digestion). Claus (1881) maintained that the ingestion of foreign corpuscles in the mesenterial filaments of cœlenterates was already described by him before Metschnikoff made mention of this fact. But in Metschnikoff's papers the phenomenon of ingestion was described in quite a convincing form, whilst Claus' previous investigations (Claus, 1874) only contained some notes on the question.

Krukenberg (1880) proved that the mesenterial filaments of actinians are organs of digestion and maintained that digestion takes place solely against these organs. According to this author the mesenterial filaments have to come in contact with the food, then a secretion of digestive fluid takes place and the food-particles are more or less dissolved (extracellular digestion)[3]. Krukenberg (1881, 1882*a*, 1882–86) was not inclined to attribute such an important function to the intracellular digestion of small particles as described by Metschnikoff. He was convinced that the filaments secrete a digestive fluid, though he was not able to demonstrate this fluid in a free state in the gastric cavity. He even expressed the opinion that the enzymes of the captured animals could be used by the actinians for their digestion (Krukenberg 1882*b*, 1882*c*).

The investigations of Willem (1892, 1893) made it probably that besides intracellular digestion also extracellular digestion of

[3] In the work of O. and R. Hertwig (1879/80) already the opinion is put forward that the mesenterial filaments secrete a digestive fluid. This was based only on the study of the histological structure of these organs. The definite proof of the digestive function of the mesenterial filaments is given by the physiological studies of Metschnikoff and Krukenberg.

the food takes place in actinians. The larger food-particles are more or less dissolved into smaller corpuscles by the secretion of a digestive fluid, and afterwards the small objects are ingested by the entoderm-cells as described by Metschnikoff. The ingestion of these small particles usually takes place in a zone of the mesenterial filaments in the neighborhood of the free edge, but after abundant feeding all the entoderm cells of the gastric cavity can ingest food, even those of the acontia. In the sea-anemone *Tealia* the soft parts of amphipods of the genus *Talitrus* are completely dissolved till only the bare skeleton is left (Willem, 1892). In siphonophores Willem (1894) found approximately the same phenomena: here also extracellular and intracellular digestion are present.

According to Chapeaux (1893) in the gastric cavity of actinians a free digestive fluid can be demonstrated. The secretion of this fluid occurs when food comes into contact with the mesenterial filaments. When the food is ingested in the entoderm cells the reactions of the food-vacuoles is acid. In siphonophores Chapeaux found that 15–20 hours after ingestion of food colored with litmus the vacuoles still had a red color. Chapeaux concluded from this fact that the digestive enzymes of actinians and siphonophores acted in an acid medium. The same opinion also is upheld by Metschnikoff (1893), Mesnil (1909) and Roaf (1910). On the other hand Jordan (1907*a*) supposed that after the acid reaction in the vacuoles an alkaline one would follow; the final resorption of the food probably would take place in the latter stage. The enzymes of the anthozoans then would present more likeness to those of other groups of invertebrates, and act in about the same way as the trypsin of vertebrates (cf. also Jordan, 1907*b*).

A number of investigations on digestion in actinians have been made to show whether there is only intracellular digestion in these animals or if there also is secreted a free digestive fluid which dissolves the larger corpuscles into small particles which can be ingested by the entoderm cells. According to Mesnil (1901) no free enzyme is secreted in the gastric cavity: the digestion in actinians is exclusively intracellular. Jordan (1907*a*), however, showed that food which could not come into contact with the

mesenterial filaments could be digested, and concluded that an enzyme causing extracellular digestion can be secreted by the mesenterial filaments. This opinion is also found in an article by Willem (1916), who maintains that extracellular digestion of the larger particles precedes the ingestion (phagocytosis) of the small particles, which are further dissolved by intracellular digestion.

The opinion of Biedermann (1911) on this question is in some respects an intermediate one between the two views dealt with above: he supposes that besides the intracellular digestion also the secretion of a digestive enzyme occurs. The reason why the enzyme is not found free in the gastric cavity (Mesnil, 1901) is according to Biedermann probably the following: only small quantities of digestive fluid are secreted by the mesenterial filaments, and only in immediate contact with the food.

Jordan (1913) upholds his original opinion, based on experiments with food packed in filter paper (Jordan, 1907*a*), which was digested in the gastric cavity of the polyps. This proves that the enzyme can easily penetrate through the whole of the food corpuscles and diffuse through every part of the gastric cavity where food particles are present. There is no special need for a contact of the food with the mesenterial filaments.

The principal organs of digestion in madreporarians are the mesenterial filaments, the same as in actinians. In *Cœnopsammia* (*Dendrophyllia*) Gardiner (1900) found a small crustacean in one of the polyps, supported by the mesenterial filaments, which indicates that it was being digested here. In the mesenterial filaments of *Flabellum* the same author (Gardiner, 1904*a*) found fat globules and diatoms and other algal matter in the cells of the portion next to the edge which is crowded with nematocysts. When remains of partially digested food were found in the gastric cavity of madreporian polyps, these were lying against the mesenterial filaments, as recorded by Carpenter (1910) for *Isophyllia* (*Mussa*) and by myself (1924) for *Favia*. Moreover strong evidence for the digestive function of the mesenterial filaments is given by Vaughan (1912), who states that these organs in *Orbicella* may protrude through the column wall and catch and digest the food outside the gastric cavity.

In other groups of Anthozoa there are also a number of statements on the digestive function of the mesenterial filaments. In *Pennatula* and *Virgularia* Marshall and Marshall (1882) found foreign bodies embedded in the cells of these organs. Wilson (1883) has observed the ingestion of food by the mesenterial filaments of *Leptogorgia*, and the same author found diatoms and other solid foreign corpuscles enclosed in the mesenterial filaments of alcyonid polyps (Wilson, 1884). The most elaborate researches on digestion in octactinians have been made on *Alcyonium*. According to Hickson (1901) the mesenterial filaments of this animal secrete a digestive fluid which dissolves the food. The latter is afterwards ingested by the entoderm of the gastric cavity. These conclusions were confirmed by Pratt (1906). After elaborate feeding experiments on *Alcyonium* and the study of the changes in histological structure before and after feeding in the polyps of this form Pratt came to the same conclusion: in alcyonid polyps extracellular as well as intracellular digestion occurs. Before feeding the gland cells in the stomodæum and in the mesenterial filaments are filled with a secretion, after feeding they are empty. Pratt concludes that this secretion is mixed with the food in the gastric cavity and causes the partial dissolution of the larger objects. Afterwards the small particles are ingested by the cells of the mesenterial filaments.

The opinion of Dantan (1921) that the digestion of antipatharian polyps is only extracellular is solely based on the histological study of the one polyp in which food-particles could be found. This observation does not give sufficient evidence for the opinion that the polyps of this group are in respect to their digestion quite different from all other anthozoans.

In the mesenterial filaments of zoanthids also foreign bodies are found embedded in the cells (McMurrich, 1889, 1899). The zoanthid polyps therefore in all probability digest their food in the same way as other anthozoans.

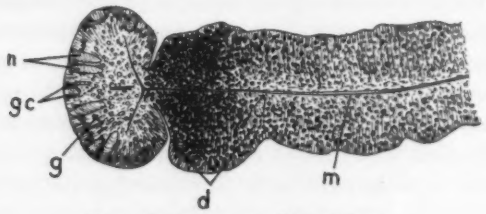
As stated above, a great number of investigators found that the mesenterial filaments are the chief organs of digestion in anthozoans. In many of these statements the accurate place of the ingestion is said to be the part immediately behind the marginal zone which contains a great number of gland cells and nemato-

cysts. The mesenterial filament then consists of a marginal, glandular part and a part with digestive function next to the marginal part. Morphologically, however, the word "mesenterial filament" usually is employed in a more restricted sense, meaning only the marginal, glandular part (cf. Schneider, 1902). Also Duerden (1902), who states that the swollen part of the mesentery next to the marginal region is generally considered to be the principal seat of digestive function, denotes the marginal region only as the mesenterial filament. I use here the term in the same sense as other authors who studied digestion in anthozoans, and differently from the definitions of Schneider and Duerden. The glandular part of the mesenterial filament contains the bifurcation of the mesoglœa. It is sharply separated from the digestive part by a deep furrow; the digestive part is, as in other madreporarians (cf. Duerden, 1902), not sharply separated from the rest of the mesentery, usually its thickness is slightly more.

In my studies on the digestion of *Astrangia* I chiefly used pounded crab meat thoroughly mixed with some coloring matter which served as an indicator after the food had been swallowed. A number of small colonies served as material for each experiment. I noted the time of feeding and studied the changes in the internal organs at various intervals by cutting open a polyp and examining a mesenterial filament under the microscope.

In the case of crab meat colored with India ink the food is readily taken by the tentacles and transferred to the mouth. Then it slowly descends through the stomodæum into the gastric cavity. By the transparency of the tissues it remains visible in the lower part of this cavity, where it is in contact with the mesenterial filaments. The polyps gradually expand more or less and often rise considerably above the skeleton (to about 15 mm., the tentacles also may stretch to the same length). When, after a very short time, the food is ingested into the mesenterial filaments these organs are visible as dark stripes through the wall of the polyps. Already one hour after feeding the digestive part of the mesenterial filament is crowded with small black spots (the particles of India ink) which indicate that the food with the coloring matter has been ingested here. Sparsely distributed black particles are also often found in the adjoining portion of the

mesentery. When food in great abundance has been offered to the polyps these parts ingest a great deal of it; when only moderate portions of food are given nearly all the food is ingested in the zone next to the marginal part. During a few days the distribution of the black particles in the mesenterial filaments does not alter perceptibly (Fig. 1); afterwards (about 5 days after



EXPLANATION OF THE TEXT FIGURE.

Transverse section of the free extremity of a mesentery of *Astrangia danæ*, circa 2 days after the feeding of the polyp with a mixture of crab meat and India ink. Fixation 5 per cent. trichloracetic acid; stained with hematoxylin Delafield, *d*, digestive region of the mesenterial filament, crowded with black particles; *g*, glandular part of the mesenterial filament; *gc*, gland cells; *m*, mesoglœa; *n*, nematocysts. × 360.

feeding) the black particles gradually disappear from the digestive part of the mesenterial filaments. On the seventh day after the feeding usually the black particles have completely vanished from the digestive organs.

Crab meat mixed with ammonia carmine gave approximately the same results: during a number of days after the feeding the digestive zone of the mesenterial filaments then contains a quantity of red vacuoles.

Another series of feeding experiments was made with crab meat mixed with litmus. This mixture keeps its blue color whilst remaining in the gastric cavity, but after the food has been ingested in the mesenterial filaments the latter acquire a red color. This change of color is clearly visible in the living animal through its transparent tissues. Microscopic examination of a mesenterial filament soon after feeding proves that in the digestive zone a large number of red vacuoles of different size are present, whilst particles of litmus lying against the mesenterial filaments but not yet ingested have retained their blue color. Consequently the

digestive vacuoles possess an acid reaction whereas in the gastric cavity this reaction is neutral (or weakly alkaline).

During two days the digestive vacuoles keep approximately the same color. After about 48 hours a few vacuoles have acquired a bluish hue. Gradually the number of the blue vacuoles increases and after about three days the majority of the vacuoles in the digestive zone of the mesenterial filaments are blue. At last there are only a few red spots left, whilst the great number of blue vacuoles remain till about seven days after the feeding. During the greater part of the time the color is evenly distributed in the comparatively large vacuoles, but towards the end of the alkaline period (on the sixth and seventh day after the feeding) the coloring matter is concentrated to small particles, which gradually disappear from the mesenterial filaments.

In the digestion of *Astrangia* we can therefore distinguish two periods: an acid one, lasting for about two days, and an alkaline period during the rest of the time. In the later part of this alkaline period the excretion of the undigestible particles occurs. The changes in the reaction of the digestive vacuoles make it highly probable that the absorption of the food takes place in the alkaline period only. The acid period then is an antiseptic one (cf. Jordan, 1907*b*), in which noxious microörganisms are killed. The authors who stated that digestion of actinians takes place in an acid medium (cf. Chapeaux, Mesnil) did not extend their experiments for a sufficient time, otherwise they probably would have found that also in actinians the acid reaction is followed by an alkaline one. As compared with protozoans these two periods last a very long time. In *Paramecium* and *Colpidium* after feeding there is an acid period of 5½ to 70 minutes which is followed by an alkaline period of 1 to 30 minutes (Nirenstein, 1905).

One of the arguments for the presence of extracellular digestion in actinians results from the experiments of Willem (1892) referred to above. In *Astrangia* I have employed approximately the same method to demonstrate the evidence for extracellular digestion. I therefore studied the digestion of large copepods by the polyps. The copepods were vitally stained with neutral red after Fischel's method. At various intervals after the feeding I

opened a polyp and noted the changes in the mesenterial filaments and in the copepods. The results were the following.

Half an hour after the feeding in the digestive zone of the mesenterial filaments a number of red vacuoles are visible. Besides these vacuoles which have a fuchsin like hue (acid reaction), the remainder of the mesenterial filament, including the marginal part, has absorbed some neutral red which is unchanged in color. The copepod still has a deep red hue.

Gradually now the intensity of the color of the mesenterial filaments increases whilst the color (together with the muscles and other soft parts) is extracted from the copepod. At first the parts of the copepod which are lying against the mesenterial filaments lose their color, the distal parts of the appendages usually keep the neutral red longer than the central part of the animal. After two hours the mesenterial filaments are strongly colored, whilst the copepod is almost completely devoid of its soft parts and only the bare skeleton is left.

When the polyps are cut open to study the changes in the food and in the digestive organs the parts of the skeleton of the copepod usually disjoint. To avoid this the experiment can be modified in some respects. When a polyp, including the skeleton, is cut longitudinally into two halves, the digestive function of the mesenterial filaments may be studied under the microscope. The skeleton prevents the tissues from contracting strongly after the dissection and the mesenterial filaments remain clearly visible.

A copepod vitally stained with neutral red placed on the mesenterial filaments soon becomes partly enveloped by a few coils of these organs and the struggling movements of the animal soon come to an end. After some time (a quarter of an hour to half an hour) these coils withdraw from the food and then the digestive zone of their surface which has been in contact with the copepod has assumed a red color, thereby forming a striking contrast with the remaining parts of the mesenterial filaments. These colored parts, then apparently saturated with food, slowly bend away from the copepod and other coils of the same or a neighboring mesenterial filament take their place. The copepod remains intact as far as the external form is concerned till almost all of the colored internal tissues have been absorbed by the mesenterial

filaments. Afterwards usually the skeleton falls into pieces but not before almost the whole of the internal organs are dissolved. After about three hours the copepod is nearly completely devoid of its soft parts and the more or less disjointed skeletal elements are removed from between the mesenterial filaments.

This experiment, which can be easily followed under the microscope from the beginning till the end, gives a strong evidence for the view that besides intracellular digestion there is in *Astrangia* a secretion of an enzyme which dissolves the food in an extracellular way. Whilst the external surface of the copepod still is completely intact the greater part of the internal organs are already dissolved and ingested by the cells of the digestive zone of the mesenterial filaments. This disintegration of the soft parts of the copepod, as long as it does not yet fall into pieces, is only possible when a digestive fluid penetrates into it.

Probably in *Astrangia* a number of different digestive enzymes may be demonstrated as in the case of other cœlenterates (cf. Bodansky and Rose, 1922). The most effective of these enzymes is undoubtedly one which is comparable to the trypsin of other animals. This we may already expect in advance as the digestive vacuoles during the later period have an alkaline reaction. Moreover in all lower animals in which the proteolytic enzymes are studied they have a trypsin-like function (cf. Jordan, 1907*b*). I have made no elaborate experiments on the nature of the enzymes in *Astrangia*, but the few enzyme preparations tested showed that a trypsin-like enzyme is the chief factor for the disintegration of the food. Owing to the polyps being small the mesenterial filaments cannot easily be separated from the other parts of the polyps. The suspensions were made by pounding the tissues (chiefly consisting of the mesenterial filaments, extracted from a number of polyps) with sand to a mash. The latter was diluted with sea water and preserved with a few drops of chloroform. To equal parts of tissue suspension a piece of crab meat (previously boiled to destroy the blood enzymes of the crab it might contain) was added. Tests, containing crab meat in sea water with chloroform, were prepared in the same time. After four days the meat was partially dissolved. No positive results were obtained with the biuret reaction; the reaction with ninhydrin,

however, gave a definite purplish blue color, proving that the liquid contained amino-acids or peptids (cf. Howell, 1922). In the tests the crab meat was practically unaltered, the liquid gave only negative results with both reagents.

The Food of Astrangia and the Symbiotic Algæ.

Among my material there were a great number of colonies the polyps of which possessed numerous zoöxanthellæ in their entoderm. The feeding of these polyps therefore can to some extent be compared with that of reef-corals, which, as a rule, also contain large quantities of these unicellular algæ. On the other hand the polyps of *Astrangia* with the symbiotic organisms can be directly compared with those of the same species which are completely free of zoöxanthellæ.

In the literature there are comperatively few notes on the food of Madreporaria. According to the statements of many authors (cf. Duerden, 1902; Gardiner, 1902–03; Pratt, 1906; Walther, 1919) only very rarely remnants of food are found in the gastric cavity of madreporarian and also of alcyonarian polyps. Now the greater part of the researches on which these statements are based were made after the study of preserved material, and as I have already pointed out before (Boschma, 1924) this is at least partially due to the contraction of the polyps in the fixing fluid. When coral-polyps strongly contract the food-remnants which are in their gastric cavity usually are discharged through the mouth.[4] In living coral-polyps from the reefs in the East Indian region I usually found remnants of food in the gastric cavity embedded in mucus.

To ascertain the natural food of *Astrangia* I now proceeded in the same way and studied the specimens in the living state. As soon as the colonies were dredged I put them in sea-water on board the ship and left them undisturbed for about a quarter of an hour. Gradually the polyps now expanded more or less. After a mechanical stimulus (a slight touch with a forceps) they suddenly contracted, and at the same time the mouth opened widely. Usually then at the bottom of the gastric cavity a slight

[4] Some years ago Dantan (1921) also tried to explain this lack of food in the gastric cavity of anthozoans by the action of the preserving fluids which cause contraction and emptying of the gastric cavity.

amount of foreign material was to be seen, which could easily be extracted with a small forceps, without any damage to the polyps. The contents of the gastric cavity of 20 polyps consisted besides of undeterminable matter (detritus) of the following foreign organisms or parts of these: living diatoms (found in 12 polyps), diatom scales (found in 9 polyps), parts of higher algæ, usually in a partially decayed state (found in 4 polyps), foraminifera (found in 1 polyp), spicules of sponges (found in 5 polyps), parts of the stalks of hydroids (found in 2 polyps), a living nematode (found in 1 polyp), a dead larva of a polychæt worm (found in 1 polyp), parts of appendages or segments of the body of different smaller crustaceans (found in 10 polyps), shells of small bivalve mollusks (found in 3 polyps). Often also nematocysts or parts of these occur among the food-remnants and in the polyps which live in symbiosis with zoöxanthellæ invariably also these yellow algæ are to be found in the gastric cavity. Only very few polyps, when examined immediately after being collected, do not contain anything in their gastric cavity.

It is an interesting fact that in those polyps of *Astrangia* in which zoöxanthellæ occur in the entoderm cells, these algæ are always found in the remains of the food in the gastric cavity. These algæ are here in different stages of decomposition, owing to their being digested by the polyps. In this respect the polyps of *Astrangia* possessing zoöxanthellæ agree closely with reef-corals, in which the symbiotic algæ are also found in a partially digested state in the mesenterial filaments.[5]

There are, in general, two opinions concerning the food of reef-corals. One of these opinions was first put forward in a number of publications by Gardiner (1899, 1902, 1902–03, 1904*b*). This author found that the zoöxanthellæ form a large proportion of the food of all reef-corals, and maintains that many species of these corals even feed entirely on their symbiotic algæ. In a later publication Gardiner (1912) states that zoöxanthellæ are largely eaten by the coral-polyps when they require food, and further mentions that it is supposed that they catch and digest the small

[5] After my studies in Woods Hole I made some observations on the feeding of a few reef corals in the Bermuda Biological Station for Research. The polyps of these corals (*Isophyllia* and *Siderastrea*) in the natural state invariably contain partially decayed zoöxanthellæ in the digestive zone of the mesenterial filaments.

organisms of the superficial water of the sea. Hickson (1906) admits that the zoöxanthellæ may constitute a part of the food of reef-corals, but thinks it improbable that there are coral-polyps that feed exclusively on their yellow cells. In a recent work of Hickson the opinion is upheld that probably "the holozoic method of nutrition of the coral is supplemented by the holophytic action of the chlorophyll-bearing zoöxanthellæ" (Hickson, 1924, p. 21).

The other opinion on the food of reef-corals is expressed by Vaughan (1912, 1919) after a great number of feeding experiments on West Indian reef-corals. His conclusion is that the food of reef-corals solely consists of animal matter. This view is also supported by Mayer (1918), who based his opinion on Vaughan's experiments and on the statement of Duerden (1904) that the polyps of *Siderastrea* are easily kept alive with meat of crabs and other animals.[6]

Vaughan's opinion was first criticized by Gravier (1913). According to Gravier it is improbable that coral-polyps live exclusively on animal matter, for their symbiotic algæ are undoubtedly also a factor in their nutrition as direct food or indirectly by the supply of carbohydrates. After the study of the contents of the cœlenteron of many coral-polyps I also (Boschma, 1924) came to a conclusion differing from Vaughan's. I found that the food-remnants in the gastric cavity of the polyps of reef-corals always contained zoöxanthellæ in various stages of disintegration, undoubtedly owing to their being digested by the polyps. Besides these algæ also animal matter was found in the remains of the food, rarely in the smaller polyps, more often in the larger polyps.

It is generally understood that the products of the photosynthesis of the zoöxanthellæ assist in the nutrition of the cœlenterates containing these algæ (Buchner 1921, 1924; Hickson, 1924). In many anthozoans which harbor large quantities of these unicellular algæ even a degeneration of the food-capturing

[6] Besides the facts on the food of reef corals there are some statements on the food of madreporarian corals which do not contain zoöxanthellæ in their entoderm. Some notes on the food of deep-sea corals are found in the publications of Gravier (1920) and Boschma (1924). In the latter article also data on the food of species of *Dendrophyllia* from shallow water are given. Cf. also Gardiner's articles on *Cœnopsammia* and *Flabellum*, cited above.

and digestive portions of the polyps has taken place, as in *Sclerophytum gardineri* (Pratt, 1903, 1906) and in *Galaxea musicalis* (Matthai, 1914).

The feeding of cœlenterates on their symbiotic algæ as a whole has been reported in a few cases only. In young medusæ of *Aurelia aurita* Friedemann (1902) has mentioned instances of this phenomenon. According to Pratt (1903) in the polyps of the alcyonarian coral *Sclerophytum* frequently zoöxanthellæ are observed in a partially digested condition in the mesenterial filaments (cf. also Pratt, 1906). Digestion of zoöxanthellæ in the polyps of reef corals is recorded by myself (1924). Probably the irregular green corpuscles which McMurrich (1889) found among the zoöxanthellæ in the digestive part of the mesenterial filaments of zoanthids were also such partially digested algæ. In *Velella* the zoöxanthellæ of the developing larvæ which migrate to deeper water probably serve as a source of food-substance only, as their photosynthetic action is impeded by the darkness (Woltereck, 1904).

According to Fulton (1921, 1922) the association of cœlenterates with their zoöxanthellæ probably is of a parasitic nature (the polyps being the parasites of the algæ), as during starvation sea-anemones feed upon the unicellular algæ rather than upon their photosynthetic products.

Besides their feeding on the zoöxanthellæ or on their products of assimilation the polyps also derive some profit from the algæ living in their tissues, as they are a source of oxygen for the polyps. The investigations of Brandt (1883), Trendelenburg (1908) and Pütter (1911) prove that actinians with zoöxanthellæ may derive a large part of their oxygen from these algæ, and that actinians with zoöxanthellæ can better resist unfavorable circumstances than those which do not harbor unicellular algæ. Probably the algæ also use nitrogenous waste products of the polyps, which may be one of the profits the zoöxanthellæ derive from the association with the cœlenterates.

In the case of *Astrangia* zoöxanthellæ are always found in the digestive region of the mesenterial filaments of the polyps which are infected with these algæ. In contradistinction to the algæ in the entoderm of the oral disk and the tentacles those in the

digestive zone have lost their yellow color to some extent. White spots then appear in their interior, whilst the contents are contracted and often have assumed a brownish hue. All different stages of the decomposition of these algæ are found in the digestive region, indicating the probability that they are being digested here. In the polyps of *Astrangia* which are not infected with zoöxanthellæ these algæ never occur in the digestive region of the mesenterial filaments. Consequently the zoöxanthellæ do not form a necessary part in the feeding of the polyps. A priori it is highly probable that they are digested on account of the lack of other food. We may therefore expect that, when food of other origin in great abundance is given to the polyps, the cells of the digestive zone of the mesenterial filaments will ingest no more zoöxanthellæ.

In fact these changes of the contents of the digestive region are easily accomplished. For my experiments I used dark brown polyps, the digestive organs of which before the artificial feeding contained a large quantity of partially decayed zoöxanthellæ. These polyps now were repeatedly fed with crab meat and already after three days the digestive region of the mesenterial filaments was almost completely devoid of zoöxanthellæ. Still a few yellow-brown corpuscles were recognizable as strongly decayed algæ, but it was evident that after the feeding of the polyps with crab meat no more algæ were ingested. The zoöxanthellæ which were already present in the digestive organs were completely digested. In this way the polyps can be compelled to change the nature of their food. It is obvious from these facts that the zoöxanthellæ are ingested by comparatively hungry polyps only. In the natural state the amount of food available for the polyps seems to be rather scanty and therefore they usually derive a part of their food from the digested unicellular algæ.

Van Trigt (1919) has described a similar phenomenon in freshwater sponges (*Spongilla*), which contain zoöchlorellæ. These unicellular algæ, which are continually imported from the surrounding water, constitute a very important source of food for the sponges. When circumstances are favorable the algæ are killed and digested by the sponge only in part, the rest of the imported algæ then can live on, photosynthesize and multiply in the tissues.

On the other hand in less favorable circumstances the whole of the imported algæ together with those already present in the tissues of the sponges are digested.

Infection of Colorless Polyps with Zoöxanthellæ.

In my experiments I kept the colonies of white polyps separated from those with brown polyps (brown on account of their zoöxanthellæ). As the white polyps did not become infected during a month's time there is little evidence that infection occurs through free zoöxanthellæ in the sea water. In a number of polyps I produced an artificial infection with zoöxanthellæ by feeding them with portions of the soft parts of dark brown polyps. Especially those parts of the brown polyps which contained a multitude of these algæ (the tentacles and the oral disk) were used for this experiment. The tissues were torn to minute pieces and thoroughly mixed with pounded crab meat. It was necessary to add meat to the tissue preparations for otherwise the polyps refused to feed upon it.

Soon after feeding a number of zoöxanthellæ had detached from the crab meat and were floating free in the gastric cavity of the polyps and sometimes even the tentacles assumed a light brown color by the algæ which had penetrated into their cavity. A large part of the zoöxanthellæ from the feeding mixture were ingested together with the meat by the mesenterial filaments. After three days the digestive region of these organs was crowded with algæ. Some of these were already partially digested, for white spots had appeared in the originally evenly yellow colored interior.

Some of the cells which a very short time after the feeding are found in the cavity of the tentacles penetrate into the entoderm cells of these organs. Here they quickly multiply by division into two halves. The following numbers clearly illustrate the quick increase in number of the zoöxanthellæ in the tentacles of recently infected polyps. In one polyp on the day after the infection a cut-off top of a tentacle contained 6 symbiotic algæ, three days after the infection a top of a tentacle of the same length contained 23. For another polyp the number of zoöxanthellæ in the entoderm of different distal parts of tentacles of about equal

size was: 1 day after the feeding 4 and 5; 2 days after the feeding 9, 16, 2, 21 and 12. In a third polyp the number of the algæ in tentacular extremities of approximately equal size was: 3 days after the feeding 30, 28, 22, 17 and 27; 9 days after the feeding 72 and 53. Consequently the zoöxanthellæ in the entoderm cells after the infection gradually had increased in number, though the change in color of the polyp could not yet be observed at first sight, for in moderately brown polyps extremities of tentacles of the same size as those cut-off in my experiments contain far more than 1,000 zoöxanthellæ.

After the feeding with the mixture of crab meat and zoöxanthellæ the infected polyps were fed several times with crab meat to keep them in a well-fed state. In hungry polyps the zoöxanthellæ perhaps might divide less rapidly.

I could not continue these experiments of infection for more than 9 days, but I am convinced that in this way a white polyp may be changed into a brown one crowded with zoöxanthellæ in the entoderm cells. Probably in nature the infection may take place (though rarely) in a similar way. When the polyps capture an animal which contains zoöxanthellæ some of these may find their way to the entoderm of the tentacles or the oral disk. After division these cells may infect the neighboring cells and at last the whole of the entoderm of the colony. This manner of infection in all probability only rarely occurs. As in other coral polyps with symbiotic algæ the greater part of the infected polyps of *Astrangia* undoubtedly have originated from planulæ which already obtained the symbiotic algæ before hatching. This view is supported by the fact that all the infected polyps of the Woods Hole region were found in a comparatively restricted locality.

Summary.

The feeding reactions of *Astrangia* in general consist of four actions: muscular action of the tentacles, muscular action of the central part of the oral disk, secretion of mucus by the oral disk, and ciliary action of the stomodæum.

The reaction of the food-vacuoles in the digestive region of the mesenterial filaments immediately after the feeding is acid; after about two days the reaction changes to alkaline. In this alkaline

period in all probability the digestion takes place. There is sufficient evidence that besides intracellular digestion a secretion of a digestive fluid (a trypsin-like enzyme) occurs.

In the polyps of *Astrangia* which contain zoöxanthellæ in their entoderm these unicellular algæ furnish a part of the normal food of the polyps: a quantity of these algæ are digested in the mesenterial filaments.

Polyps of *Astrangia* without zoöxanthellæ can be easily infected with these algæ by feeding them with crab meat mixed with parts of the tissues of strongly infected polyps.

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A STUDY OF OXYGEN METABOLISM IN *DROSOPHILA MELANOGASTER.*

M. R. CLARE.

INTRODUCTION.

Although *Drosophila melanogaster* is uniquely favorable as material for genetical studies, relatively few physiological investigations have been conducted upon this animal. Its small size has certainly been a deterrent to such studies; yet, with suitable apparatus, this feature is unimportant. To supplement the remarkably full record we possess for its genetical behavior physiological studies are especially desirable, and an attempt is made in this paper to show the practicability of investigations into the metabolism of this fly and also to illustrate the type of results which such study can be expected to yield. The investigation was undertaken primarily to determine to what extent degree of inbreeding may be reflected in metabolism.

Measurements were made of the oxygen consumption of *Drosophila* pupæ, and these proved admirably adapted to this purpose. At no time during pupal life except at its extreme termination are results complicated by muscular movements, hence standard metabolism alone is measured. Moreover, the fly is so amenable to conditions of laboratory culture that experimental pupæ are available at all times and can be grown under standard conditions.

The investigation was conducted at the University of Pennsylvania, for which privilege the writer desires to acknowledge his indebtedness to Doctor C. E. McClung. He is under special obligations to Doctor J. H. Bodine, who not only suggested the problem but was ever ready with helpful suggestions throughout the progress of the work. The stocks of experimental flies were kindly contributed by Doctors C. B. Bridges, H. J. Muller, L. E. Griffin, Chas. Zeleny, J. H. Bodine and R. L. King.

MATERIAL AND METHODS.

Eight stocks of "wild" *Drosophila melanogaster* were employed in the study. Three of these were caught shortly before the

work was undertaken in localities removed from centers where flies are grown. These may be called the "non-inbreds." The remaining five stocks had been inbred for a number of years before being received and, as some of these are well known, the following table of sources may be of interest.

TABLE I.

SHOWING DERIVATION OF EXPERIMENTAL MATERIALS.

NON-INBREDS.

Designation.	Source.	Captured.
G.....	Portland, Oregon	August, 1923
H.....	Hellam, Penna.	July, 1923
B.....	Rockaway, N. J.	July, 1923

INBREDS.

Designation.	Original Name.	Source.	Inbred Since
C–2.....	"Ossining"	Columbia University	1921
C–1.....	"Pt. Pleasant"	Columbia University	1921
F.....	"Florida No. 5"	University of Texas	1918
I.....	——	University of Illinois	1916
P.....		University of Penna.	Many years

Owing to some preliminary difficulties, work was not begun until December, 1923, or until the non-inbred stocks had become inbred for several generations. Thereafter readings were continued, with interruptions, until December, 1924, when the experimental work was concluded. The chronological distribution of the work is without significance to our study and will not be entered into.

The pupæ of *Drosophila melanogaster* are so small that it is impracticable to make an extensive series of measurements of oxygen consumption on single pupæ, hence lots of 10 or fewer pupæ were used for each determination. Readings were taken over a period of 4 or 5 hours each day throughout the duration of pupal life. Data were collected for 160 lots of pupæ.

The stock flies were grown in mass culture in large quinine bottles and kept at room temperature. The experimental pupæ, however, were always the products of single matings. From time to time the flies in a culture bottle were removed and matings were made up from new flies as they appeared which

were never more than 20 hours old. The pairs of flies were cultured in shell vials (about 9 cm. long by 2 cm. diameter) containing banana agar and were transferred each day to fresh vials so that the pupæ forming in a particular vial resulted from eggs deposited therein on a single and known day. A complete cultural record was kept which included figures for the sex ratios of the flies appearing in all of the vials in order that a check might be had on any conditions of metabolism attributable to sex peculiarities of the matings. It happened, however, that for all of the matings the distribution of the sexes remained normal. Some of the matings were cultured at variable room temperature, ranging from 21° C. to 25° C. about a mean of 23° C.; others were cultured in an incubator at a constant temperature of 25° C. It is necessary to stress this distinction, for upon it will be based a natural division of the data into two parts. Hereafter, the pupæ formed at room temperature will be referred to as of the "first experimental period," whereas those formed at 25° C. will be referred to as of the "second experimental period."

The banana-agar was prepared according to the usual method and while still liquid about 5 or 6 cc. of the material were introduced into each previously sterilized vial, which was provided with a cotton plug. Usually a sufficient number of vials was prepared at a time to supply requirements for two or three days and kept in a refrigerator while awaiting use. Before introducing a pair of flies into a fresh vial, a small amount of powdered Magic Yeast was dusted on the surface of the culture medium and on this was placed a disc of towel paper cut somewhat smaller than the bore of the vial. When a very limited amount of paper is placed in a vial, the larvæ developing therein pupate on the glass without "spinning" and therefore require a minimum of cleaning in preparation for use. They can then be removed quite readily from the glass without danger of injury with a small brush after a preliminary wetting with water.

Whenever possible, the first 10 pupæ appearing in a vial were used for a determination, but quite frequently only a smaller number could be secured. Each evening the vials for the several matings were examined and any pupa which had appeared unduly early was checked with a wax pencil in order that it

might not be included among the experimental pupæ selected the following morning. Accordingly, the maximum age of pupæ on which determinations were made was 15 or 16 hours, a point which must be kept in mind.

In preparation for the first reading a lot of pupæ was first washed in water with a camels' hair brush, then treated with 80 per cent. alcohol for 2 or 3 minutes to destroy any adhering yeast cells, rinsed in water and dried on filter paper. After being weighed on a delicate balance they were placed in a cotton-lined basket and suspended in the oxygen-measuring apparatus. Between determinations each lot of pupæ was kept in an individual moist chamber with a piece of moist filter paper. Weighings as well as determinations were continued each day until development was so far advanced that there was danger of flies emerging. As a final step, record was made of the sexes of the flies which issued from each lot of pupæ.

Rates of oxygen consumption were measured with an improved form of the manometer of Krogh (1915), described by Bodine and Orr (1925). Six manometers were used, a single one only being used for a given lot of pupæ during the period of pupal life. During readings the manometers were placed in a water bath which was kept at a constant temperature. From day to day, however, the temperature of the bath varied in accord with the temperature of the room, but this fluctuation is not registered in the determinations as oxygen values are always reduced to 0° C. Calculation of rates has been made on the basis of oxygen consumption per minute of time and both per gram body weight and per single pupa.

The "Oxygen Curve."

The duration of pupal life is influenced largely by temperature. An index of this correspondence is afforded by figures for the number of days on which oxygen determinations were possible—even though such figures do not represent the actual duration of pupal life. During the first experimental period 4-day pupæ were predominant, 5-day pupæ occurring rarely and 3-day pupæ only to the extent of 7 per cent. At a temperature of 25° C., on the other hand, the percentage of 3-day pupæ was increased to 60 per cent., while 5-day pupæ did not occur.

During the course of pupal development certain readjustments are in progress which are reflected in rates of metabolism. In Fig. 1 the changes in metabolism are shown in the form of curves. Those for 4-day pupæ are derived from a single mating of the second experimental period while those for 5-day pupæ pertain to the first period. Each curve shows an initial fall after the first day of pupal life succeeded by an abrupt or gradual rise. The most instructive are the curves for 5-day pupæ in which the period of depression of metabolic rate is seen to continue from the second to the third day. The curves for 4-day pupæ are obviously abbreviations of these.

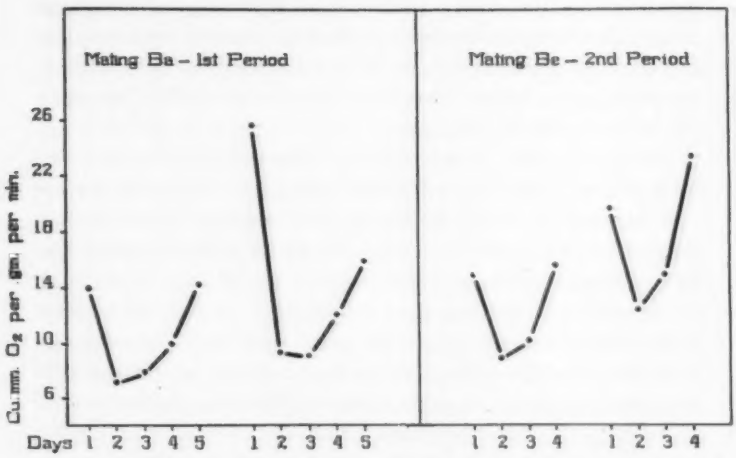

FIG. 1. Metabolism curves based on O_2 consumption per minute per gram of body weight. Ordinates = O_2 values in cubic millimeters; abscissæ = time in days.

This type of curve appears to be characteristic of pupæ in general, the modifications presented in the several species that have been investigated being due chiefly to the varying extensions of the period of depression. The significance of this type of pupal curve has been discussed at length by several workers among whom may be mentioned Tangl (1909, 1 and 2), Weinland (1906), and quite recently Fink (1925). The researches of Weismann, Perez, and others have demonstrated that early in pupal life the persisting larval tissues undergo a series of histolyses

leading to their ultimate dissolution, and that the tissues of the imago are built up through the activity of certain groups of cells which survive histolysis and appear to be set aside for this specific purpose. In other words, two distinct processes are in progress during pupal life—a destructive and a constructive, the latter being inaugurated before the completion of the former. The authors cited identify the abrupt fall in metabolism early in pupal life with the histolytic process and the recovery after the period of depression with the formation and growth of imaginal tissues.

Variability in Rates of Metabolism.

When a series of curves for rates of oxygen consumption is examined, a feature which is most striking is the considerable variability in values exhibited. The majority of the values for any one day of pupal life fall within fairly narrow limits, but scattered among these are numerous others representing very high as well as rather low rates, distributed in a seemingly erratic manner. An early examination of the data proved that most of the very high rates belonged to lots of pupæ, one or more members of which not only failed to give issue to flies but failed to pass beyond the stage of development characteristic of second day pupæ. Instances of this sort suggest that probably intestinal microörganisms find their capacity for growth released by the death of the pupa or pupæ harboring them, and by their rapid multiplication elevate the rate of oxygen consumption to a high level. With one exception to be considered later, it was deemed necessary to completely eliminate from further consideration all data pertaining to lots of pupæ showing incomplete development, thereby reducing the total number of determinations by about one third. Several further eliminations are for lots of pupæ which were accidentally shaken from their supports within the manometers into the 2 per cent. NaOH solution over which they were suspended. Although washed as quickly as possible in a large volume of water and again set up in the manometer, a lot so treated almost invariably responded with an abnormal elevation of rate. Mention should also be made of a series of determinations which was set aside because of bacterial contamination

of the cultures. This condition resulted in the production of a limited number of very small pupæ of exceptionally low weights. A single example from this series has been retained in Table 5 in order to show that, in spite of the very low pupal weights, the rate of metabolism is to all appearances normal. The rather drastic elimination which has been practiced has reduced to a considerable degree both the number of determinations and the variability in rates, but that the latter has been by no means removed is shown in Fig. 2.

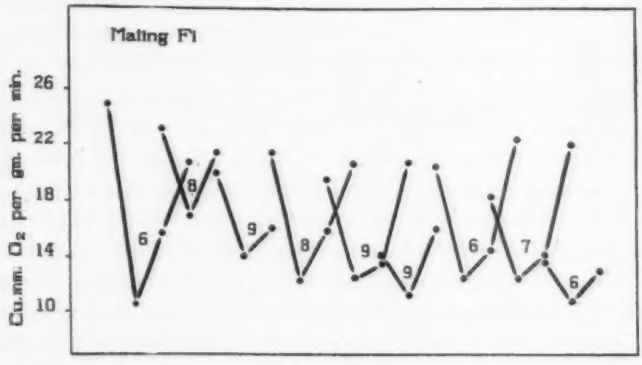

FIG. 2. Metabolism curves showing variability in rates within a single mating, cultured at 25° C. Numbers indicate particular manometers used in establishing rates. Ordinates = O_2 per gram per minute, in cu. mm.

There are several conceivable factors which might underlie this variability and they will be taken up in turn. It is obvious that if comparisons are to be made between the several stocks in respect to oxygen consumption this irregularity must be reduced to a minimum. Suspicion was at once cast on the calibration of the manometers used in making the determinations. That these are not the primary source of the variability will be evident from a further consideration of Fig. 2. The curves of this figure are for lots of pupæ all of which are offspring of a single pair of flies. Associated with each curve is a number which is that of the manometer used in determining the rates. For each of two manometers, numbers 6 and 9, three sets of determinations are represented which, it should be noted, exhibit marked variability in rates of metabolism. The manometers were very carefully

calibrated several times and any possible errors from this source, although carefully looked for, have eluded detection.

Pupal Weights and Metabolism.

In studies on respiratory metabolism the practise has been to emphasize the relation between weights of experimental organisms and intake of oxygen or production of carbon dioxide, on the assumption that for comparable samples of living material within a species, the rates of metabolism remain fairly constant. Rubner, along with others, on the other hand, has instituted the procedure of basing metabolism on extent of body surface, arguing, in the words of Krogh (1916) that "the metabolism is simply a function of the conditions for loss of heat, while there is no such thing as a specific oxidative activity of the cell." Rubner later saw fit, however, to qualify this idea. These practises were derived from investigations on warm-blooded animals possessed of a heat-regulating mechanism, and their applicability to invertebrate animals is extremely uncertain. Unfortunately, the studies on metabolism in invertebrates have been so few in number and have afforded results of so conflicting a character that conclusions based on them do not seem to be justified. We feel obliged, therefore, to examine the data for *Drosophila* with some care and determine, if possible, the significance to be attached to weight; and attempt to decide whether or not rates of metabolism are subject to decided change. No data are available on which to base figures for pupal surface.

Pupal weights exhibit a wide range in value, the means for newly-formed or first-day pupæ in lots of 10 varying between 11 and 15 milligrams. At the extremes of the range lots of 10 pupæ may possess a weight as low as 9 mg. or as high as 17 mg. For the products of a single mating, likewise, the range of variability is very pronounced.

In addition to the rôles played by food and overcrowding as factors affecting pupal weight—and these can hardly be considered as applying in this work—temperature certainly is a determining agency. An examination of Table 2 shows that pupal weights of the second period are significantly lower than those for the first period. In the summer of 1923, during a

TABLE II.

PUPAL WEIGHTS AND OXYGEN PER GRAM FOR TOTAL STOCKS.

Figures for weights are based on lots of 10 pupæ, stated in milligrams; figures for oxygen consumption are rates per minute, stated in cubic millimeters.

	1st Day.	2d Day.	3d Day.	4th Day.
FIRST PERIOD—4-DAY PUPÆ.				
Weights	14.04 ± .20	12.57 ± .17	12.46 ± .17	12.36 ± .18
O_2 per Gram	15.89 ± .34	9.00 ± .15	10.38 ± .21	15.44 ± .42
SECOND PERIOD—4-DAY PUPÆ.				
Weights	13.30 ± .18	11.87 ± .15	11.78 ± .17	11.65 ± .18
O_2 per Gram	18.35 ± .44	11.00 ± .20	13.30 ± .35	21.15 ± .35
SECOND PERIOD—3-DAY PUPÆ.				
Weights	13.45 ± .18	12.25 ± .14	12.06 ± .14	
O_2 per Gram	17.94 ± .38	12.28 ± .28	16.71 ± .36	

period when the room temperature varied between 25° and 30° C., a collection of weights was made which for lots of 10 pupæ present mean values of 11.83 ± .152, 10.86 ± .169 and 11.00 ± .194 for the three days of pupal life. It is, therefore, evident that an inverse relation obtains between temperature and pupal weight. When the range of temperature is not very great,

TABLE III.

COEFFICIENTS OF VARIATION FOR PUPAL WEIGHTS AND OXYGEN PER GRAM.

The coefficients are based on the figures presented in Table II.

	1st Day.	2d Day.	3d Day.	4th Day.
FIRST PERIOD—4-DAY PUPÆ.				
Weights	11.2 ± 1.03	10.4 ± .97	10.4 ± .97	10.7 ± 1.02
O_2 per Gram	16.5 ± 1.51	13.0 ± 1.22	15.1 ± 1.41	20.3 ± 1.93
SECOND PERIOD—4-DAY PUPÆ.				
Weights	9.8 ± .97	9.1 ± .90	10.3 ± 1.02	10.2 ± 1.09
O_2 per Gram	17.3 ± 1.72	13.1 ± 1.30	18.5 ± 1.84	10.9 ± 1.16
SECOND PERIOD—3-DAY PUPÆ.				
Weights	11.3 ± .94	9.9 ± .83	9.4 ± .80	
O_2 per Gram	18.3 ± 1.52	19.2 ± 1.62	17.9 ± 1.53	

however, this correlation does not manifest itself when curves for temperature and pupal weights are compared. Moreover, a constant temperature serves merely to limit somewhat the range of fluctuation, instead of insuring stability, as can be seen from Table III. Temperature, then, operates only in a large way in its influence on weight of pupæ.

Corresponding to the sudden drop in metabolism shortly after the inauguration of pupal life, there occurs a marked drop in pupal weight. Sometimes this loss is relatively enormous, in other exceptional cases relatively insignificant. Unlike metabolism, however, there is no recovery in weight after the second day, the level for the third and fourth days remaining approximately identical with that for the second day. Sometimes for these latter days a slight increase is registered but as frequently a slight fall occurs.

The interesting sequence of changes during the pupal period in respect to pupal weights and metabolism is presented in Fig. 3. The data on which this figure is based represent a mating selected for illustration because a large number of determinations is available and the pupal weights show an unusual consistency of level. The figure is divided into parts corresponding to the several days of pupal life and a common scale is used which enables one to carry out comparisons between any two days.

The relationship between the curves for metabolism per pupa and per unit body weight affords an index of the ratio existing between weight of respiring tissue and that of inert materials associated with the respiring tissue. When the two curves follow a strictly parallel course this ratio must remain constant in the several lots of pupæ, provided complicating factors are not present. When for a particular determination the curves become approximated, the inference may be made that the relative amount of non-respiring or inert materials is reduced, whereas, when they become more widely separated, this condition may be attributed to a relative increase in the non-respiring materials present. It will be clear, therefore, that in general the ratio of respiring to non-respiring substances in the pupæ on which Fig. 3 is established remains a fixed quantity. Interesting exceptions, however, appear in the first and last determinations, numbers 8

and 37. In the latter the relation of the metabolism curves for the first day indicates that the excessive weight of the pupæ in question was due to the presence of a disproportionately great amount of non-respiring material, let us suggest water. As pupal development proceeded, this disproportion gradually became

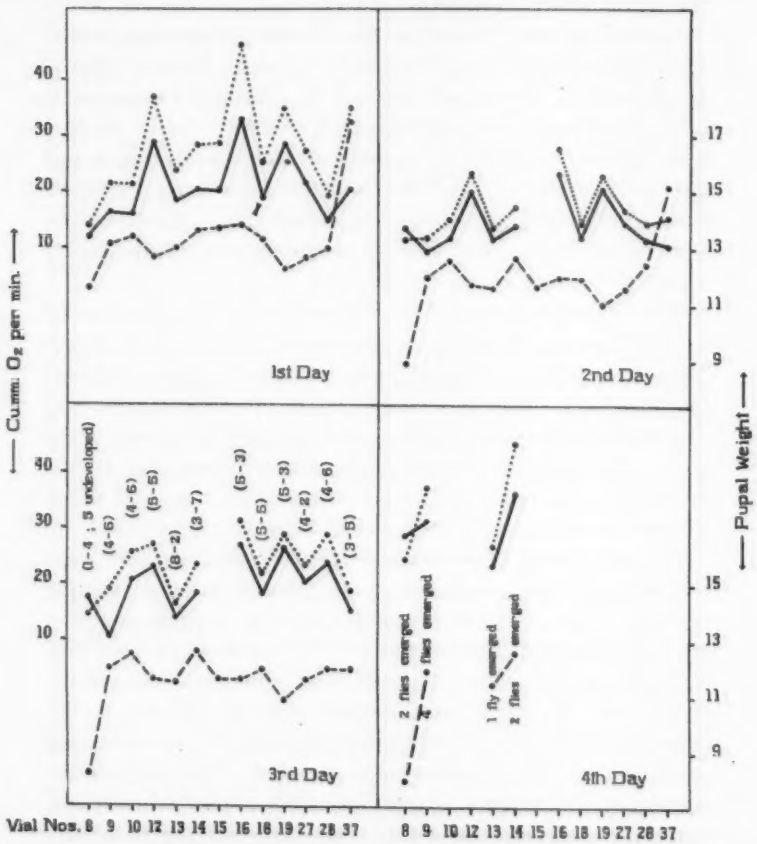

FIG. 3. Modifications in metabolic rates and pupal weights during pupal life for lots of pupæ derived from a single mating, C–2:4, at 25° C. Corresponding values for each day of pupal life are connected into curves. Upper curve = O_2 per pupa; middle curve = O_2 per gram and lower curve = pupal weights. Ordinates at left are O_2 values in cu. mm., at right are pupal weights in milligrams per 10 pupæ. Abscissæ are numbers of the vials from which experimental pupæ were obtained. Sex-ratios in brackets (males—females).

reduced until, on the third day, the normal balance of respiring and non-respiring materials was restored. The first determination, number 8, on the other hand, presents a different story which is complicated by the fact that 5 of the 10 pupæ on which this determination is based failed to complete their development, the only case of the sort we shall consider. The approximation of the metabolism curves suggests that the pupæ contained a very small amount of non-respiring material relatively to the respiring tissues, yet this idea does not seem to harmonize with the remarkable fall in pupal weight occurring after the first day of pupal life. If, however, we postulate for this case, as was done earlier for cases of arrested development in general, a bacterial activity which would serve to elevate the metabolism per unit body weight relatively to the total metabolism, we develop an interpretation which affords an understanding not only of the peculiar relation shown by the two curves but also of the cause for the unusual decrease in pupal weight. Instances of this sort, representing a fluctuation in the relative amounts of respiring and non-respiring substances present in pupæ, are of frequent occurrence and are further illustrated in Fig. 5. The only general statement that seems permissible is that pupæ of very low weight usually show an elevation of the rate per unit weight relatively to the total metabolism but this condition is by no means invariably true. It appears, therefore, that mere weight is a rather unreliable index of the amount of respiring tissue.

It is manifest that for the first and second days of pupal life a rough proportionality certainly exists between pupal weights and metabolism, thus explaining in a way the trends of the oxygen curves. However, the peculiarities of the 12th, 16th and 19th determinations must receive an interpretation of their own. As the two metabolism curves remain closely parallel, the only conclusion that seems justifiable is that the rates for the determinations in question are of a discontinuous type; in other words, independent of pupal weight. It would appear, therefore, that strictly comparable samples of respiring tissue grown under, and subjected to, like environmental conditions can nevertheless exhibit marked diversity in rates of metabolism. It is ex-

ceedingly improbable that the independent rates of these determinations are due to a secondary source, bacterial for example, contributing in an additive manner to the respiring pupal tissues. If this were the case, we should expect the condition to be indicated by the metabolism curves.

As applied to *Drosophila*, the practice of bringing metabolism into relation with pupal weight is warranted only in a most general way. The correlation is most pronounced during the first day of pupal life when the coefficient of correlation for 4-day pupæ grown at 25° C. attains a value of 0.47 ± .071. On the second and subsequent days this value is considerably reduced. Too much importance should not be attached even to this correlation. The relationship often fails completely in individual cases, as is shown in Fig. 5. This fact, taken in connection with the rather frequent tendency toward the establishment of discontinuous rates, indicates that an understanding of metabolism in *Drosophila* is not to be sought on the basis of weight of respiring tissue but rather through the impress of factors regarding whose nature we are at present in ignorance.

A point of considerable interest brought out in Fig. 3 and in other figures for similarly prepared material is the constancy of the relation between the respiratory rates for the individual lots of pupæ up to the third day of pupal life. The striking similarity of the metabolism curves for the first and second days of pupal life indicates that the rates for this period are relatively stable and that the establishment of independent rates may occur either before or later but hardly during this period. On the third day, however, after the inauguration of growth and differentiation of imaginal tissues, a new order of rates is ushered in, which is that of adult life. As the metabolism curves for the third day in Fig. 3 show, these new rates considered in their entirety still preserve to a slight degree their kinship with those of early pupal life, but each lot of pupæ now develops along a new course. It appears that the rates of first-day pupæ in no way can serve to forecast those of the flies which will later emerge from them. We have already suggested that the period of institution of new adult rates is a somewhat critical stage in pupal development. The great majority of pupæ of arrested

development, apart from those containing fully formed flies unable to escape from the pupal cases, represented the stage of development characteristic of 2-day-old pupæ. In other words, the histolytic process had been gone through but the reconstructive had failed of attainment.

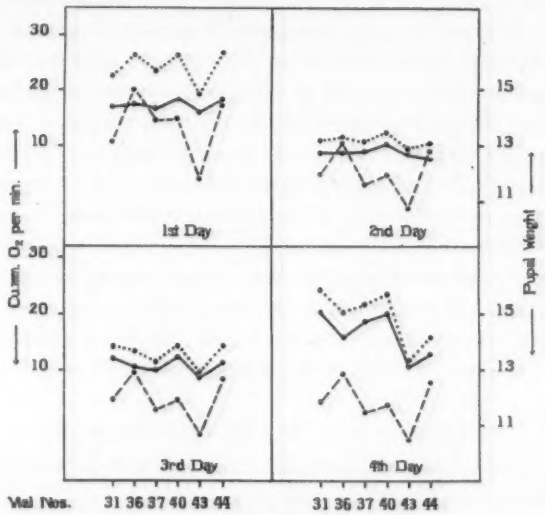

FIG. 4. Metabolic rates and pupal weights for pupæ of the first experimental period, products of single mating Hd. Upper curve = O_2 per pupa; middle curve = O_2 per gram; lower curve = pupal weights. Ordinates = O_2 values in cu. mm. at left, pupal weights in mg. per 10 pupæ at right. Abscissæ = numbers of the vials from which experimental pupæ were obtained.

Do flies about to emerge possess a higher standard metabolism than do larvæ? Our selected figure throws some light on this question. The elevated rates for the four-day pupæ in the figure are certainly in large part to be explained as due to muscular contractions incident to emergence of the flies, and accordingly these records do not indicate standard metabolism. When we recall that the figures for the first day of pupal existence are for pupæ which may be as old as 15 or 16 hours and in which histolysis and its accompanying depression in rate of metabolism has perhaps been in progress for an unknown length of time, it would appear from a comparison of the rates for the first and

third days of pupal development that the standard metabolism of larvæ is actually somewhat greater than that of the flies although, relatively to body weight, the two stages may be more nearly comparable.

Before turning from the general subject we have been considering, a point of difference between the metabolism curves for the first and second experimental periods should be noted. For a cultural temperature of 25° C., we have seen that marked fluctuations occur in rates of oxygen consumption among lots of pupæ derived from a single mating, and corresponding in a very rough way with variations in pupal weight. In material representing the first experimental period at a lower temperature, the extent of fluctuations in metabolism is less pronounced until the last day when it becomes increased. Pupal weights of the first period are more irregular than for the second period and in consequence the parallelism between weight and metabolism tends to become lost. The relationship between pupal weight and metabolism for this period is represented in Fig. 4.

Influence of Sex on Metabolism.

It has been pointed out that for any one day of pupal life the metabolic rates for different samples of pupæ vary considerably and that weight is a very untrustworthy guide in arriving at an understanding of this irregularity. Is sex a contributing factor? Biological literature abounds in references to the physiological distinctness of the sexes and several important recent researches have depended for interpretation on the postulate that male animals possess a higher rate of metabolism than do female. So far as the writer knows, only one experimental attempt has been made to measure this supposed difference directly, namely, the investigation by Benedict and Emmes (1915). With human subjects these workers found a slight increase in metabolism in favor of males over the rates for females.

There are certain facts at hand to suggest a possible difference in metabolism between the sexes of *Drosophila*. At times, at least, there is a well developed tendency for female flies to emerge earlier than the males from the first-formed pupæ. The present data happen to be inconclusive on this point. The

TABLE IV.

SEX AND PUPAL WEIGHTS.

Pupal weights are stated in milligrams for lots of 10 pupæ.

First Day.

♂♂	Pupal Weights.										♀♀
	8	9	10	11	12	13	14	15	16	17	
1											9
2							×	14.30 ± 1.187			8
3							×	14.37 ± 0.780			7
4						×		13.35 ± 1.630			6
5						×		13.90 ± 1.337			5
6					×			12.87 ± 2.152			4
7					×			12.79 ± 1.778			3
											2
9											1

Second Day.

	8	9	10	11	12	13	14	15	16	17	
1											9
2						×		12.90 ± 1.300			8
3						×		13.00 ± 0.223			7
4					×			12.08 ± 1.245			6
5					×			12.38 ± 1.132			5
6				×				11.64 ± 1.679			4
7				×				11.58 ± 1.418			3
8											2
9											1

Third Day.

	8	9	10	11	12	13	14	15	16	17	
1											9
2											8
3					×			12.66 ± 0.145			7
4					×			12.00 ± 0.107			6
5					×			12.31 ± 0.175			5
6				×				11.54 ± 0.278			4
7				×				11.72 ± 0.213			3
8											2
9											1

records do, however, suggest a tendency for larvæ destined to become females to pupate earlier than those which will develop into males. Also, as is shown in Table IV., pupæ which are to become females incline toward heavier weight than those which become males.

The sex ratios for a typical series of determinations have been indicated in Fig. 5 as well as in Fig. 3. All of the data have

been gone over most carefully with the unqualified result that sexual differences in metabolism are impossible to detect. If such exist they must be so small that they are obscured by irregularities induced by other agencies.

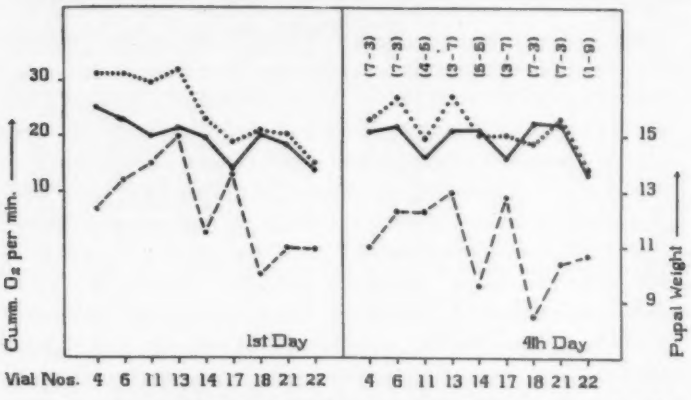

FIG. 5. Oxygen rates, pupal weights and sex-ratios for lots of pupæ of mating Fi, of the second experimental period. Curves for the second and third days of pupal life are omitted. Sex-ratios shown in brackets, the first figure for males, the second for females. Upper curve = O_2 per pupa; middle curve = O_2 per gram; lower curve = pupal weights. Ordinates at left are O_2 values in cu. mm., at right are pupal weight values in mg. per 10 pupæ. Abscissæ refer to the vials from which experimental pupæ were obtained.

FURTHER CONSIDERATIONS ON IRREGULARITY IN RESPIRATORY RATES.

The most significant result which this study has revealed is the peculiar irregularity in rates of metabolism exhibited especially by pupæ formed under conditions of constant temperature. It might be remarked that there is no ground for believing that the causes underlying this irregularity are genetic in character. On the contrary, the usual tendency of the rates for lots of pupæ derived on consecutive days from a common source to exhibit a graduated character either in the direction of elevation or of depression strongly suggests the effect of graduated environmental influences. We have already eliminated pupal weight and sex as important agencies responsible for irregularity in rates and we may now refer to several remaining possibilities.

It is believed that under the conditions of the experiment food conditions were maintained as nearly uniform as is possible. Moulds occasionally became established in the culture vials but the growth rarely if ever became noticeable until after the larvæ had pupated. It is altogether possible that organisms harmful to the larvæ and of sporadic occurrence in the tubes may have contributed in slight measure to the irregularity, but this is hardly a likelihood. We have referred to the bacterial contamination of one series of cultures, with the consequent reduction in weight of the pupæ. Curiously enough, this reduction in weight does not appear to have induced a parallel modification in rate of metabolism.

The pupæ of *Drosophila*, it would seem, are adapted to resist conditions of desiccation, at any rate for short periods. On several occasions lots of pupæ were inadvertently subjected overnight to the drying effect of the room air. This treatment produced no detectable effect on the following day either in pupal weight or in rate of oxygen consumption. This point is of some interest in connection with the results obtained by Caldwell (1925), who has found that the metabolism of some animals, as measured by carbon dioxide production, is modified as a result of desiccation.

A degree of irregularity in oxygen rates was certainly introduced with varying ages of the pupæ used. Larvæ developing from eggs deposited on a single day possess individual peculiarities, some pupating precociously, others delaying the act for a day or longer after their brothers and sisters have made the change. Accordingly, all of the pupæ thrown together for a determination are not of the same age. Among lots of experimental pupæ, however, the mean ages should be nearly identical as selection was performed at about the same hour each morning and the manometer readings were distributed over approximately identical time periods each day.

Mention might be made of an attempt to find an interpretation of the irregularity in rates through a study of varying intensities of such environmental factors as light, humidity, etc., data for which were secured from the local weather office. As might be expected, this attempt was not fruitful of results. Likewise, a

careful examination of data on length of larval life, of pupal life, etc., proved barren of results, except that a suggestive but very rough correspondence between rates of oxygen consumption and productivity, as measured by the number of flies appearing in the vials, did come to light. A correspondence of this sort would carry the implication that the metabolic rates of the pupæ are due to an impress set by the metabolic condition of the female parent at or about the particular time the eggs are deposited, and that this impress continues without impairment over the period of larval life. The data at hand do little more than suggest this possibility.

Effects of Inbreeding.

The central question around which this study was planned was whether or not it is possible to find an index of inbreeding in rates of metabolism. If metabolism were subject to control as a result of genetic make-up, we should expect to find evidence of it reflected in our results. There is not only no indication that heredity plays any but the most general rôle in metabolism; but, on the contrary, we have seen some reason for believing that rates are at the command of graduated environmental influences. The irregularity in rates which we have stressed renders it impossible to make satisfactory comparisons between different matings within a common stock, and the propriety of lumping all of the matings of a stock under a mean is very questionable. However, this has been done in Table 5 in which are presented means for most of the stocks. The eliminations previously referred to made such serious inroads on the data for the majority of the matings that Table V. must be built up on an inadequate number of determinations. The figures for stock G of the first period are based on as few as 3 determinations; and in all other cases on 4 determinations except where a probable error is attached, this indicating that 5 or more determinations were available. Deficient as it is, this table illustrates the impossibility of separating inbred from non-inbred stocks on the basis of rates of metabolism.

Summary.

Several results emerge from this study which, it is believed, should assist in defining the problem and, at the same time,

TABLE V.

PUPAL WEIGHTS AND OXYGEN PER GRAM (IN CU. MM. PER MINUTE) FOR INDIVIDUAL STOCKS.
Non-inbred stocks are starred (*).

Stocks.	1st Day.		2d Day.		3d Day.		4th Day.	
	Weight.	O_2 per G.	Weight.	O_2 per G.	Weight.	O_2 per G.	Weight.	O_2 per G.
FIRST PERIOD—4-DAY PUPÆ.								
H*	14.25 ± .31	17.12 ± .42	12.62 ± .16	9.50 ± .18	12.62 ± .16	10.62 ± .29	12.62 ± .16	15.75 ± .73
G*	13.00	11.50	12.50	8.50	12.50	11.00	12.50	15.50
P	15.40 ± .15	15.20 ± .48	13.80 ± .12	8.60 ± .31	13.40 ± .24	10.20 ± .22	13.25	15.70
I	14.33	15.25	12.66	8.00	12.66	9.00	12.33	12.66
C–2	12.20 ± .35	16.40 ± .84	10.50	9.50	10.25	10.70	10.25	15.20
C–1	14.66	17.33	13.00	9.66	13.00	11.00	13.00	17.33
SECOND PERIOD—4-DAY PUPÆ.								
H*[1]	11.71	17.42	10.71	9.71	10.40	11.75	10.66	17.00
G*	13.75	17.50	12.50	10.00	12.25	12.50	12.25	20.50
I	14.25	14.75	12.25	9.25	12.25	11.50 .	12.25	18.25
C–2	13.60 ± .15	19.60 ± .67	12.20 ± .12	12.00 ± .38	12.20 ± .12	14.00 ± .63	12.00	23.50
F	12.60 ± .33	19.30 ± .74	11.30 ± .28	11.60 ± .24	11.20 ± .34	14.60 ± .46	11.10 ± .31	22.10 ± .29

[1] The low weights of the pupæ of stock H of the second period were due to pollution of the cultures by bacteria.

should suggest types of investigation which are most likely to contribute to an understanding of metabolism in *Drosophila melanogaster*.

It has been shown that a close approach to a knowledge of metabolism in *Drosophila* pupæ cannot be made if sole dependence is placed on weight of respiring tissues as a guide. In a very rough way, pupal weights show a correspondence with the trends of metabolic rates, but, for particular matings, especially when the larvæ are maintained at lower temperatures, the correspondence is largely lost. It has also been shown that rates of oxygen consumption are very irregular and vary in a manner highly suggestive of the influence of environmental agencies. The not infrequently pronounced elevations above the general level of metabolism lend support to the conclusion that the metabolic rates for comparable samples of respiring tissue are not necessarily fixed within narrow limits. Finally, it has proved impossible to find a metabolic difference between the sexes or to detect any difference in metabolism between inbred and non-inbred stocks.

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THE ACTION OF ETHER ON PROTOPLASM.[1]

L. V. HEILBRUNN.

No problem in general physiology has been investigated more frequently than the problem of anesthesia. Many workers have attempted to discover the essential nature of the action of anesthetics on living substance. Not only have animals, tissues, and cells been studied, but some physiologists have even gone so far as to study the effect of anesthetics on lifeless materials which they believed similar to protoplasm.

Many theories have been proposed to explain anesthesia. In recent years the permeability theory has had a wide following. This theory claims that anesthetics either decrease the permeability of the cell, that is to say of the plasma membrane, or that they at any rate prevent an increased permeability. The actual evidence in support of the theory is somewhat scanty. What there is has been well summarized by Winterstein (1), Höber (2), and Lillie (3), all three of whom are strong advocates of the theory.

In only a few cases has it been shown that anesthetics lower cell permeability and most of these cases are doubtful, as Winterstein admits. In some instances observers have claimed that anesthetics produce an increase rather than a decrease in permeability (see Höber). Perhaps the work most often quoted in support of the doctrine that anesthetics lower permeability is the series of conductivity measurements of Osterhout (4) on plant cells. There is however a growing realization that a decrease in the electric conductivity measurements of a group of cells does not necessarily depend on a decrease in the permeability of the plasma membranes of these cells. Many factors play a part in influencing the conductivity measurement. When an electric current is sent through a mass of cells, certainly a large part of the current goes between and around the cells. Changes in the conductivity of the spaces between cells are therefore apt

[1] Contribution from the Zoölogical Laboratory of the University of Michigan.

to be of greater moment than changes in the conductivity of the cells themselves. The factors involved include possible changes in the size and shape of the spaces between cells, as well as changes in their conductivity. One such factor is perhaps of especial importance for substances like the ordinary fat-solvent anesthetics which alter the viscosity of the medium in which they are dissolved. This is the effect of a change in the viscosity of a solution on its conductivity. Concerning this effect Walker (5) says: "The addition of a small quantity of a substance such as alcohol to water increases the viscosity of the water. Corresponding to this increase we find that the rate of diffusion is less when a substance is dissolved in water containing a little alcohol than the rate of diffusion when water alone is the solvent, no matter what the dissolved substance may be. Similarly the speed of ions in water containing alcohol is less than their speed in pure water."

Let us consider some of Osterhout's experiments more closely. He measured the conductivity of Laminaria in various solutions of anesthetics and compared it with the conductivity of the same material in sea-water. In one experiment he adds to 970 cc. sea-water 10 cc. of ether plus about 5 cc. of sea-water concentrated by evaporation until its conductivity was about double that of ordinary sea-water. In another experiment he adds to 970 cc. sea-water 30 cc. of absolute alcohol plus 15 cc. of concentrated sea-water (apparently made up as before), thus obtaining a 0.5 molecular solution of alcohol which he refers to as 0.05 molecular. This solution, according to Osterhout, has the same conductivity as sea-water. But this scarcely seems possible. Osterhout's solution has approximately the same concentration of salts as sea-water, but the viscosity of the solution is decidedly higher. Pissarjewsky and Karp (6) found that a 0.5 molecular concentration of alcohol lowers the conductivity of normal NaCl solution until it is about 8 per cent. below that of the conductivity of normal NaCl solution in pure water. On the other hand, the ether solution used by Osterhout would have, as he claims, approximately the same conductivity as sea-water, for Arrhenius (7) found that 1 per cent. ether lowers conductivity only about 2 per cent. for various types of electrolytes.

But there is also another important fact to be considered. Separating the cells of *Laminaria* is a network of cell walls. This is composed of cellulose and cellulose-like material which offers little resistance to dissolved substances and is obviously one of the main paths for an electric current. The adsorptive powers of cellulose are well known, although it apparently adsorbs electrolytes much more readily than non-polar compounds. In the finely divided condition in which the cellulose occurs in the cell walls it is not improbable that substances of low surface tension like ether, alcohol, and chloroform would be selectively adsorbed on it and more or less concentrated there. We should at least be led to expect this from Gibbs' adsorption equation. Moreover the surfaces of the cells would also tend to adsorb the anesthetic. If such adsorptive processes occur, then the conductivity of the cellulose framework as well as the conductivity of the cell surfaces would doubtless be decreased, and the decrease in conductivity would be greater than that which occurred in the mass of the solution. If this is true then it might in itself account for the variations in conductivity found by Osterhout.

It is interesting to note that the experiments of McClendon (8) on the diffusion of electrolytes from anesthetized pike eggs also fail to take cognizance of the direct effect of the anesthetic on the diffusion rate. It might be thought that the difference in diffusion rate would also explain McClendon's experiments. This is not the case. The presence of 2 per cent. alcohol would lower the diffusion rate less than 8 per cent. McClendon apparently found a lowering of as much as 50 per cent. in the total amount of chlorides diffused from the eggs. But his results are not as trustworthy as it might at first sight be thought. McClendon determined the diffused chlorides nephelometrically as silver chloride, precipitating them with silver nitrate. Now the precipitation of silver chloride varies under diverse conditions; there is a well known tendency for it to go into colloidal solution. The presence of alcohol either alone, or in conjunction with the albuminous substances also present, might act as a peptizing agent and hinder the precipitation of the silver chloride. Alcohol occasionally acts as a peptizing agent, and the precipitation of

silver chloride is known to be hindered by at least one non-electrolyte (see Bancroft (9), pp. 167, 168). Richard and Wells (10) in their first description of the nephelometer stated that "care must be taken to have both standard solution and unknown solution subjected to precisely the same conditions, for varying conditions of precipitation may lead to differences in the appearance of the precipitate far greater than the possible optical error of the apparatus. Herein lies the chief caution to be noted in its use." Later this point was emphasized again by Richards (11), who points out that "if even moderately accurate analytical results are to be had with the nephelometer, the one essential point to be heeded is this: *the unknown solutions to be estimated must be treated in exactly the same way as the known standard solutions, which serve as the basis for comparison.*" This precaution was neglected by McClendon and his results are therefore unreliable.

McClendon's work and that of Osterhout constitute a large portion of the evidence presented by Winterstein and regarded by him as trustworthy evidence in favor of the permeability theory. It would seem therefore that the support of the theory does not always rest on very solid ground.

Most of the earlier work on permeability change during anesthesia concerned itself with the attempt to demonstrate a change in permeability toward dissolved substances. More recently both Winterstein (12) and Lillie (13) have independently shown a change in permeability to water. I have repeated both sets of experiments, although in the former case I performed only a few tests and my results can scarcely be considered as constituting either a proof or a disproof of Winterstein's views. Fig. 1 shows two examples of the entrance of water into normal and anesthetized muscle as shown by the increase in weight. The abscissas represent time, the ordinates the weight of the muscle. The muscle used was the gastrocnemius muscle of the frog, and a pair of muscles from a single animal was used in each experiment.

The curves show that the entrance of water is faster in the normal muscle than in the anesthetized muscle. Winterstein obtained a somewhat more striking difference; he used the sartorius muscle rather than the gastrocnemius.

As to the interpretation of the experiments, the difference in rate of osmotic flow may be due either to a change in the properties of the fluid or to a change in the plasma membranes. Bigelow (14) and Bartell (15) found that the speed of osmotic

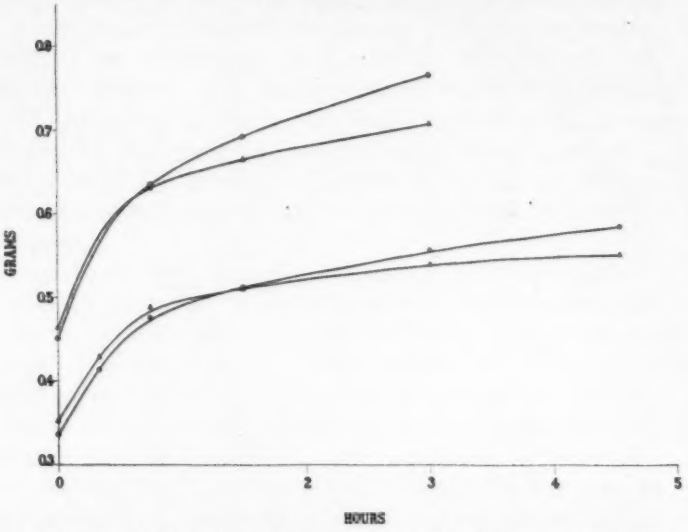

FIG. 1 shows the increase in weight of muscles in distilled water and in distilled water to which 2 per cent. by volume of ether has been added. In each pair of curves the small circles represent the increase in weight of a frog gastrocnemius muscle in distilled water, the small triangles represent the increase in weight of the corresponding muscle from the other leg immersed in 2 per cent. ether. The abscissæ indicate time in hours, the ordinates total weight of muscle.

flow of water through various osmotic membranes followed the laws of Poseuille for flow through capillary tubes. It is obvious that in general any increase in the viscosity of a fluid would tend to slow its rate of osmotic flow. The magnitude of the effect might be sufficient to account for my results, although perhaps not for those of Winterstein. There are also other factors to be considered. The anesthetics may extract materials from the muscle which tend to exert osmotic pressure in the opposite direction.

I have thus far omitted reference to Winterstein's experiments on the osmotic flow through muscle membranes obtained from

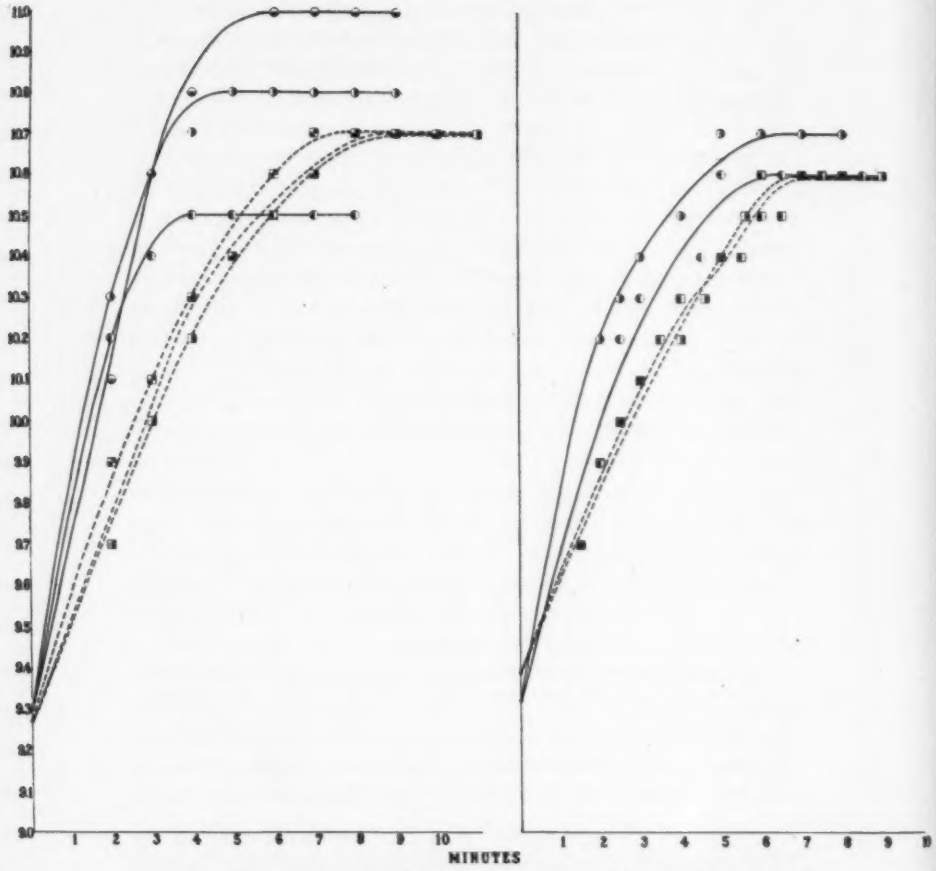

FIG. 2. In the above figures the curves represent increase of diameter of individual sea-urchin eggs in equal parts of sea-water and distilled water. (The original size is an average measurement.) The continuous lines represent etherized eggs, the broken lines control eggs, not exposed to ether. The concentration of ether used was 1 per cent. by volume, and the eggs were first treated with 1 per cent. ether in sea-water and were then transferred to 1 per cent. ether in equal parts of sea-water and distilled water. The ordinates represent the diameter of the eggs in arbitrary scale units, the abscissæ show the number of minutes elapsed after the entrance of the egg into the hypotonic solution. Similar results were also obtained with 2 per cent. ether solutions, but the measurements are harder to make as the eggs frequently rupture.

the body wall of female frogs. These experiments constitute the greater portion of Winterstein's evidence and the reader is referred to his paper for details. As I understand the experiments, Winterstein measured the osmotic flow into short glass cylinders sealed at *both* ends with muscle membranes. In such a system it would seem that the entrance of water would depend more on the distensibility of the muscle membranes than on their permeability. The high concentration of alcohol and other anesthetics used by Winterstein might tend to coagulate the membranes and make them less distensible. This would simulate a decreased permeability. However I have not worked with this sort of a set-up and I am not sure that I can judge it properly.

My results with sea-urchin eggs are perhaps more interesting. With this type of material Lillie found that the shrinkage of the cell in hypertonic solutions was delayed by the presence of ether in anesthetic concentration. This may very well be true, but it need not be due to a change in permeability to water. For it is easy to show that when eggs are placed in hypotonic solutions, they expand just as readily, or even more readily in the presence of ether than in its absence. This is illustrated in Fig. 2, which compares the expansion of etherized and unetherized eggs in hypotonic solutions. The shrinkage and expansion of a cell depends on many factors. Of these permeability is one, but not the only one. Other factors of importance are surface tension, the rigidity of the plasma membrane, and the colloidal condition of the interior. This last factor is of especial importance in Lillie's experiments. Lillie found that after fertilization the sea-urchin eggs showed a greater tendency to crenation and shrinkage when placed in hypertonic solutions, and that this increased tendency to crenation was prevented by anesthetics. As a matter of fact fertilized eggs become somewhat crenate even without being placed in hypertonic solutions (16). The crenation is concomitant with, and doubtless dependent on, the gelation of the protoplasm which follows fertilization. In view of the fact that anesthetics such as ether prevent this gelation (see below), it is easy to see that they would tend to hinder crenation.

Finally it should be pointed out that the experiments of Loeb (17) apparently indicate an *increase* in the permeability of *Fundulus* eggs to water in the presence of various anesthetics.

It is not my purpose to claim that in anesthesia there is no decrease in the permeability of the cell to salts or to water. Perhaps eventually this may be found to be a constant occurrence. But it seems at least premature to conclude a decreased permeability from the slight and uncertain evidence hitherto presented. Only by closing our eyes to the experiments that show the opposite of a decrease and only by neglecting the numerous sources of error which have scarcely been considered by previous experimenters, is it possible to consider the permeability evidence as convincing.

Even should we, some time in the future, find it true that anesthesia is always associated with decreased permeability of the plasma membrane of the cell, we would not be very much closer to an understanding of how and why the anesthetic stops cell activity. Permeability is a surface phenomenon, the activity of the cell goes on largely in the interior. Thus even if we accept the permeability doctrine, we must postulate a secondary hypothesis to explain how the permeability effect is transferred to the interior of the cell.

Many years before the permeability doctrine was thought of, various physiologists held the idea that anesthetics affected the colloidal condition of the protoplasm in one way or another. For a long time this idea remained a mere speculation, but in recent years it has been definitely shown that anesthetics do have a very real effect on the colloidal state, or at any rate upon the viscosity of the protoplasm. Both in plant and animal cells it has been shown that dilute solutions of ether cause a liquefaction of the protoplasm, and that more concentrated solutions cause a coagulation. There is a remarkable correspondence between widely different sorts of living substance. However in plants only those concentrations of ether which cause coagulation prevent the rotary movement of the protoplasm. This was therefore regarded by Heilbronn (18) as the anesthetic concentration. In animal cells on the other hand it was found that concentrations which caused liquefaction prevented cell-

division and therefore acted as anesthetic (16). As a matter of fact there is no difficulty in reconciling the two sets of observations. For it seems certain that what is anesthetic for mitosis is not necessarily anesthetic for protoplasmic streaming. An increased fluidity of the protoplasm would doubtless increase the speed of streaming, and yet such a cell with rapidly flowing protoplasm would scarcely be able to undergo a mitotic division and would therefore be anesthetized so far as cell-division was concerned. This is essentially the view taken by Weber (19) who points out that there is full accord in the investigation of both plant and animal cells.

Doubtless protoplasmic activity involves frequent changes in colloidal condition. Presumably any agent which prevents such changes in colloidal condition without causing death may act as an anesthetic. If this view is correct then either an increased liquefaction or a coagulation of the protoplasm may result in anesthesia. This idea was presented a number of years ago, and it was pointed out that there may very well be two kinds of anesthesia (20).

In earlier work it was claimed that those solutions of ether which act as an anesthetic for the process of cell-division in the sea-urchin egg cause a liquefaction of the protoplasm (16, 20). More concentrated solutions were found to cause a coagulation which was irreversible. All these facts have recently been called into question by Chambers (21). He claims that anesthesia in the sea-urchin egg is accompanied by an *increase* rather than a decrease in viscosity and that this condition of increased viscosity is reversible. Chambers' opinion is based on observations of Brownian movement as well as on a study with the microdissection apparatus.

It is evident that the findings of Chambers are in direct opposition to the earlier work mentioned above. An attempt was therefore made to repeat the older observations with the centrifuge method to determine if perhaps an error may not have been involved. In this repetition an effort was made to obtain more nearly quantitative results.

Experiments were performed both with fertilized and unfertilized eggs. We will consider the unfertilized eggs first.

When (unfertilized) sea-urchin eggs are placed in 2½ per cent. or 3 per cent ether, there is, within a few minutes, a very pronounced decrease in viscosity as shown by tests with the centrifuge. In the following table the viscosity of etherized and normal control eggs is compared. The viscosity numbers represent the number of seconds of centrifugal treatment necessary to produce a given degree of granular movement. Usually the eggs were centrifuged until the clear hyaline zone extended along one third or one half of the axis of the egg. Naturally a number of tests had to be performed to obtain any given value. The centrifugal force employed was approximately 4,968 times gravity and the centrifuge used was an ordinary hand centrifuge with hæmatocrit attachment. Details of technique are given in earlier papers (16, 22). Because of the fact that a series of tests had to be performed for each value of the viscosity and because moreover the viscosity of the etherized protoplasm is not constant, the tests had to be made hurriedly. They are not extremely accurate, and it is entirely possible that the inaccuracy amounts to 10 or 20 per cent. The decided difference between unetherized and etherized eggs makes this inaccuracy of small consequence. In the table the first column gives the percentage of ether used (volume per cent. in sea-water). The second column represents the time of exposure to ether, that is to say the number of minutes elapsed after immersion in ether solution at the time the critical test was made. The third column gives the relative viscosity of the etherized eggs as compared to that of the normal control eggs shown in the fourth column. The temperature of the experiments varied from 22 to 25 degrees. It is given in the fifth column.

Per Cent. Ether.	Exposure, Minutes.	Viscosity Etherized Eggs.	Viscosity Control Eggs.	Temperature.
2.5	11	10	25	23°
2.5	4	15	25	
2.5	8	15	25	22
2.5	10	15	28	24
3	15	15	35	22
3	8.5	20	40	23
3	3	15	30	25.3

Averaging the above values it is seen that in 2½ per cent.

ether the viscosity of the protoplasm is 53 per cent. of that of the normal control eggs in sea-water. The viscosity of the eggs in 3 per cent. ether is only 48 per cent. of that of the untreated eggs.

It must not be supposed that the viscosity of the protoplasm of etherized eggs remains constant for long periods. In the 3 per cent. solution the viscosity became lower and lower until finally a minimum was reached. Then a sudden coagulation occurred. This is shown by a series of tests on eggs in 3 per cent. ether (temp. 22.0°). When these eggs were tested after an exposure of 3 minutes it was found that centrifugal treatment for 15 seconds produced only a slight hyaline zone. The same results were obtained after 6 and 10 minutes, but after an exposure of 15 minutes, similar centrifugal treatment resulted in the formation of a hyaline zone which extended along about one third of the axis of the egg. After an exposure of 20 minutes the same treatment resulted in a hyaline zone which filled nearly half of the egg. On the other hand after 26 minutes when eggs were centrifuged for 15 seconds, although some eggs showed a hyaline zone extending through half of the egg, others showed no movement of granules at all. The protoplasm in these eggs is completely coagulated. Thus in 3 per cent. ether at 22° the liquefaction of the protoplasm is soon followed by a solidification.

The question may now be asked as to which of these two conditions represents a condition of anesthesia. In my earlier paper I had claimed that only the fluid state was anesthetic and that gelation or coagulation following ether treatment resulted only in death. But Chambers states that not the fluid but the gel state is the anesthetic condition when eggs are subjected to ether. On reading Chambers' statement I was at first led to suppose that I had been mistaken in making my claim too forcibly. I thought perhaps that although it was certain that moderately long exposure to ether after coagulation had taken place was lethal, nevertheless it might be true that Chambers had removed the eggs immediately after coagulation had begun and that in this instance the coagulative action of the ether was reversible.

The question was soon put to the test. In the experiment

cited above it was pointed out that coagulation occurred after 26 minutes exposure to ether. In this same experiment some of the eggs were removed from the ether solution after an exposure of 24 minutes and placed in normal sea-water. Others were removed from the ether after an exposure of 28 minutes. Some of the eggs exposed to ether 24 minutes were inseminated, following an interval of 18 minutes after removal from the ether, and some of the eggs exposed 28 minutes were inseminated following an interval of 14 minutes after removal from ether. None of the inseminated eggs showed any signs of development. As a matter of fact both the inseminated eggs and those not exposed to sperm went through the same series of degenerative changes. All of them disintegrated by breaking up into small globules.

Thus it is obvious that following the coagulative action of ether there is no recovery. The same sort of experiment was repeated a number of times always with the same result. If eggs are to recover from ether treatment they must be removed from the ether solution some few minutes before coagulation has begun.

The discussion so far has been concerned only with conditions in unfertilized eggs. In fertilized eggs the effects of ether are even more pronounced. Let us consider a sample experiment. In the following account many details of observation are omitted.

July 22 (Temp. about 24°). Eggs were fertilized at 3.35 P.M. Fifteen minutes later, at 3.50 P.M., they were centrifuged at the usual rate for 50 seconds. No zones appeared (control unfertilized eggs showed a hyaline zone about ¼ of the distance along the egg axis after 30 seconds treatment). At 3.52 P.M. some of the fertilized eggs were placed in 2½ per cent. ether in sea-water in a glass-stoppered weighing bottle. At 3.55 P.M. a centrifugal test for 60 seconds showed only a thin streak for a hyaline zone in the control untreated fertilized eggs. At 4.27 P.M. the etherized eggs when centrifuged for 20 seconds showed a hyaline zone extending more than a third of the distance along the egg axis. At 4.31 P.M. a test of the etherized eggs for 15 seconds showed a similar zone extending about one third of the axis, and at 4.33 P.M. a 10 second test showed a hyaline zone extending through approximately one fourth of the egg.

From this experiment we can conclude that when fertilized eggs are placed in 2½ per cent. ether at a time when the viscosity of their protoplasm is at its height, the ether reduces the viscosity to less than one sixth of its original value. Another experiment of the same sort may also be cited.

July 23 (Temp. 23°). Eggs were fertilized at 10.45 A.M. At 11.00 A.M. some of the fertilized eggs were put into 2½ per cent. ether in a glass-stoppered weighing bottle, others of the same lot were centrifuged for 60 seconds. The centrifugal treatment for 60 seconds resulted in a faint indication of a hyaline zone. At 11.05 A.M. a centrifugal test of the normal fertilized eggs for 80 seconds showed a hyaline zone extending about one fourth of the egg axis. Test samples of etherized eggs were centrifuged for 5 seconds at 11.12 A.M., for 10 seconds at 11.18 A.M., and for 15 seconds at 11.23 A.M. The 5 second test showed only faint indications of a hyaline zone, the 10 second test showed the zone not very plainly, the 15 second test showed it extending along one third of the axis of the egg. Later a 10 second test, at 11.35 A.M., showed the hyaline zone extending for ¼ to ⅓ of the egg axis. This test is not as trustworthy as the earlier tests, for the protoplasm appeared to be in chunks, and it is possible that the granules were fusing together. At 11.40 P.M., a 15 second test showed the hyaline zone ⅓, ¼, or absent. Coagulation is doubtless beginning.

This experiment showed that 2½ per cent. ether may lower the protoplasmic viscosity to one sixth or even one eighth of its original value in fertilized eggs.

As with the unfertilized eggs the protoplasm of the etherized fertilized eggs becomes more and more fluid with increase in ether concentration or length of exposure until suddenly coagulation occurs. Here too the onset of coagulation results in death. If the eggs are removed from the ether in the early stages of liquefaction they resume their development, but if they are kept in ether until coagulation has occurred or is about to occur, the eggs are permanently injured and never resume development.

The result of these experiments is a direct confirmation of my older results. These findings do not agree with the statement of Chambers previously referred to. Let us consider this statement closely. He says:

"Both A. Heilbronn and Weber agree with the coagulation theory of narcosis. On the other hand, L. Heilbrunn (1920) claims that the reversible effect of 2.5 per cent. ether on the sea-urchin egg occurs only when the viscosity is diminished. With higher concentrations of ether (3 per cent. +) the increased viscosity, according to him, is irreversible. He therefore concludes that narcosis implies a diminution in viscosity of the protoplasm. My results with the micro-dissection method and by observing Brownian movement do not agree with this. In 2 per cent. ether, Brownian movement was slowed down but

did not cease, and cleavage delayed but not stopped. In 2½ per cent. ether, cleavage was stopped and, both by means of the needle and by the cessation of Brownian movement, this was shown to be accompanied by a decided increase in viscosity (cf. p. 300)."

The first sentence of the above quotation is not quite true. As already pointed out Weber's ideas on ether narcosis agree exactly with mine. But this is a minor point. The essential fact is that Chambers has apparently obtained actual experimental results in direct contradiction with mine. How can this be interpreted?

In a previous paper I have already taken occasion to criticize the microdissection method for its over-great subjectivity (22), and this criticism has been supported by Heilbronn (23). But Chambers claims that his results were obtained both by microdissection and by the observation of Brownian movement. For some time I was puzzled as to how to explain the difference in his findings. Finally the idea suggested itself that Chambers in his studies of Brownian movement may have subjected the eggs to heat. It has already been shown that heat and ether act together, so that in low concentrations of ether, heat coagulation is hastened (24). Such coagulation is moreover reversible. On questioning Chambers it was found that in his experiments an arc lamp was used for illumination and no special precaution was taken to eliminate the heat factor. Here then is an explanation of the divergent results obtained by the Brownian movement method. As for Chambers' results with the microdissection method, these are probably not very trustworthy, for he was doubtless influenced by the results of his Brownian movement investigations. Another worker, Hyman, also using the dissection method, has roughly confirmed my views concerning the effects of ether on the protoplasm of sea-urchin eggs (25). Miss Hyman used ordinary steel needles instead of a microdissection apparatus.

Summary.

1. Water enters etherized muscle less rapidly than normal muscle, but this does not necessarily imply a change in the properties of the plasma membrane following etherization.

2. Ether does not lower the permeability of sea-urchin eggs to water.

3. Dilute ether solutions cause a very sharp decrease in the viscosity of sea-urchin egg protoplasm, both in fertilized and unfertilized eggs. Rough quantitative measurements of this decrease are given.

4. Slightly more concentrated solutions of ether produce a coagulation which is irreversible.

5. The divergent results of Chambers find a simple explanation.

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